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(54) Title: METHODS OF TREATMENT WITH COMPOUNDS HAVING RARα RECEPTOR SPECIFIC OR SELECTIVE ACTIVITY

(57) Abstract

Retinoid compounds which act specifically or selectively on RARα receptor subtypes in preference over RARβ and RARγ receptor subtypes, possess desirable pharmaceutical properties associated with retinoids, and are particularly suitable for treatment of tumors, such as acute monocytic leukemia, cervical carcinoma, myeloma, ovarian carcinomas and head and neck carcinomas, without having one or more undesirable side effects of retinoids, such as inducement of weight loss, mucocutaneous toxicity, skin irritation and teratogenicity.

1 METHODS OF TREATMENT WITH COMPOUNDS HAVING RAR$_\alpha$

2 RECEPTOR SPECIFIC OR SELECTIVE ACTIVITY

3 BACKGROUND OF THE INVENTION

4 1. Field of the Invention

5 The present invention relates to the use of

6 compounds which have specific or selective agonist

7 like activity on RAR$_\alpha$ retinoid receptors for

8 treatment of diseases and conditions which respond

9 to treatment by such retinoids. More particularly

10 the present invention is directed to the use of RAR$_\alpha$

11 receptor specific or selective agents for the

12 treatment of tumors.

13 2. Background Art

14 Compounds which have retinoid-like activity are

15 well known in the art, and are described in numerous

16 United States and other patents and in scientific

17 publications. It is generally known and accepted in

18 the art that retinoid-like activity is useful for

19 treating animals of the mammalian species, including

20 humans, for curing or alleviating the symptoms and

21 conditions of numerous diseases and conditions. In

22 other words, it is generally accepted in the art

23 that pharmaceutical compositions having a

24 retinoid-like compound or compounds as the active

25 ingredient are useful as regulators of cell

26 proliferation and differentiation, and particularly

27 as agents for treating skin-related diseases,

28 including, actinic keratoses, arsenic keratoses,

29 inflammatory and non-inflammatory acne, psoriasis,

30 ichthyoses and other keratinization and

31 hyperproliferative disorders of the skin, eczema,

32 atopic dermatitis, Darriers disease, lichen planus,

33 prevention and reversal of glucocorticoid damage

34 (steroid atrophy), as a topical anti-microbial, as

35 skin anti-pigmentation agents and to treat and

2

1   reverse th effects of age and photo damage to the
2   skin. Retinoid compounds are also useful for the
3   prevention and treatment of cancerous and
4   precancerous conditions, including, premalignant and
5   malignant hyperproliferative diseases such as
6   cancers of the breast, skin, prostate, cervix,
7   uterus, colon, bladder, esophagus, stomach, lung,
8   larynx, oral cavity, blood and lymphatic system,
9   metaplasias, dysplasias, neoplasias, leukoplakias
10 and papillomas of the mucous membranes and in the
11 treatment of Kaposi's sarcoma. In addition,
12 retinoid compounds can be used as agents to treat
13 diseases of the eye, including, without limitation,
14 proliferative vitreoretinopathy (PVR), retinal
15 detachment, dry eye and other corneopathies, as well
16 as in the treatment and prevention of various
17 cardiovascular diseases, including, without
18 limitation, diseases associated with lipid
19 metabolism such as dyslipidemias, prevention of
20 post-angioplasty restenosis and as an agent to
21 increase the level of circulating tissue plasminogen
22 activator (TPA). Other uses for retinoid compounds
23 include the prevention and treatment of conditions
24 and diseases associated with human papilloma virus
25 (HPV), including warts and genital warts, various
26 inflammatory diseases such as pulmonary fibrosis,
27 ileitis, colitis and Krohn's disease,
28 neurodegenerative diseases such as Alzheimer's
29 disease, Parkinson's disease and stroke, improper
30 pituitary function, including insufficient
31 production of growth hormone, modulation of
32 apoptosis, including both the induction of apoptosis
33 and inhibition of T-cell activated apoptosis,
34 restoration of hair growth, including combination

3

1   therapies with the pres nt compounds and other

2   agents such as Minoxidil[R], diseases associated with

3   the immune system, including use of the present

4   compounds as immunosuppressants and

5   immunostimulants, modulation of organ transplant

6   rejection and facilitation of wound healing,

7   including modulation of chelosis.

8   United States Patent Nos. 4,740,519 (Shroot et

9   al.), 4,826,969 (Maignan et al.), 4,326,055

10   (Loeliger et al.), 5,130,335 (Chandraratna et al.),

11   5,037,825 (Klaus et al.), 5,231,113 (Chandraratna et

12   al.), 5,324,840 (Chandraratna), 5,344,959

13   (Chandraratna), 5,130,335 (Chandraratna et al.),

14   Published European Patent Application Nos. 0 170 105

15   (Shudo), 0 176 034 A (Wuest et al.), 0 350 846 A

16   (Klaus et al.), 0 176 032 A (Frickel et al.), 0 176

17   033 A (Frickel et al.), 0 253 302 A (Klaus et al.),

18   0 303 915 A (Bryce et al.), UK Patent Application GB

19   2190378 A (Klaus et al.), German Patent Application

20   Nos. DE 3715955 A1 (Klaus et al.), DE 3602473 A1

21   (Wuest et al., and the articles J. Amer. Acad. Derm.

22   15: 756 - 764 (1986) (Sporn et al.), Chem. Pharm.

23   Bull. 33: 404-407 (1985) (Shudo et al.), J. Med

24   Chem. 1988 31, 2182 - 2192 (Kagechika et al.),

25   Chemistry and Biology of Synthetic Retinoids CRC

26   Press Inc. 1990 p 334 - 335, 354 (Dawson et al.),

27   describe or relate to compounds which include a

28   tetrahydronaphthyl moiety and have retinoid-like or

29   related biological activity.

30   United States Patent Nos. 4,980,369, 5,006,550,

31   5,015,658, 5,045,551, 5,089,509, 5,134,159,

32   5,162,546, 5,234,926, 5,248,777, 5,264,578,

33   5,272,156, 5,278,318, 5,324,744, 5,346,895,

34   5,346,915, 5,348,972, 5,348,975, 5,380,877,

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1   5,399,561, 5,407,937, (assigned to the same assignee
2   as the present application) and patents and
3   publications cited therein, describe or relate to
4   chroman, thiochroman and 1,2,3,4-tetrahydroquinoline
5   derivatives which have retinoid-like biological
6   activity.
7   United States Patent No. 4,723,028 (Shudo),
8   Published European Patent Application Nos. 0 170 105
9   (Shudo), German Patent Application No. DE 3524199 A1
10 (Shudo), PCT WO 91/16051 (Spada et al.), PCT WO
11 85/04652 (Polus) and J. Med Chem. 1988 31, 2182 -
12 2192 (Kagechika et al.), describe or relate to aryl
13 and heteroaryl or diaryl substituted olephines or
14 amides having retinoid-like or related biological
15 activity.
16   United States Patent Nos. 4,992,468, 5,013,744,
17 5,068,252, 5,175,185, 5,202,471, 5,264,456,
18 5,324,840, 5,326,898, 5,349,105, 5,391,753,
19 5,414,007 and 5,434,173 (assigned to the same
20 assignee as the present application) and patents and
21 publications cited therein, describe or relate to
22 compounds which have retinoid-like biological
23 activity and a structure wherein a phenyl and a
24 heteroaryl or a phenyl and a second phenyl group is
25 linked with an olephinic or acetylenic linkage.
26 Still further, several co-pending applications and
27 recently issued patents which are assigned to the
28 assignee of the present application, are directed to
29 further compounds having retinoid-like activity.
30   It is now general knowledge in the art that two
31 main types of retinoid receptors exist in mammals
32 (and other organisms). The two main types or
33 families of receptors ar respectively designated
34 RARs and RXRs. Within each type there are subtypes;

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in th RAR family the subtypes are designated RAR_{α},
RAR_{β} and RAR_{γ}, in RXR the subtypes are: RXR_{α}, RXB_{β} and
RXR_{γ}. It has also been established in the art that
the distribution of the two main retinoid receptor
types, and of the several sub-types is not uniform
in the various tissues and organs of mammalian
organisms.

It is also known in the art that the use of
retinoid-like compounds for the treatment of various
diseases and conditions is not without problems or
side effects. The side effects at therapeutic dose
levels include headache, teratogenesis,
mucocutaneous toxicity, musculoskeletal toxicity,
dislipidemias, skin irritation, headache,
hepatotoxicity, etc. These side effects limit the
acceptability and utility of retinoids for treating
disease. Research is still ongoing in the art to
determine which of the RAR or RXR familes and within
each family, which of the subtype or subtypes are
responsible for mediating certain therapeutic
effects, and which type or subtypes are responsible
for mediating one or more of the undesired side
effects. Accordingly, among compounds capable of
binding to retinoid receptors, specificity or
selectivity for one of the main types or families,
and even specificity or selectivity for one or more
subtypes within a family of receptors, is considered
a desirable pharmacological property. Such
selectivity or specificity is useful as a research
tool for discovering the roles of the several
receptor types and subtypes in mediating the various
effects of retinoids in biological systems, and also
as aid for designing retinoid drugs with specific
therapeutic effects and/or with reduced side effects

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1 and toxicity. Along thes lines, United States

2 Patent No. 5,324,840 describes a class of compounds

3 in which retinoid-like activity is accompanied by

4 reduced skin toxicity and reduced teratogenic

5 effects. United States Patent No. 5,399,586

6 describes the use of compounds having RXR retinoid

7 receptor agonist activity for the treatment of

8 mammals afflicted with tumors. United States Patent

9 No. 5,455,265 describes methods of treatment of

10 mammals with compounds having agonist-like activity

11 on RXR receptors. Published PCT application No.

12 WO93/11755 is also directed to the use of compounds

13 which are selective RXR receptor agonists.

14   The present invention provides methods of

15 treatment of tumors with compounds which are

16 specific or selective to RAR_α receptors.

17   **SUMMARY OF THE INVENTION**   It has been

18 discovered in accordance with the present invention

19 that retinoid-like compounds which act selectively,

20 or preferably even specifically on RAR_α receptor

21 subtypes in preference over RAR_β and RAR_r receptor

22 subtypes, possess desirable pharmaceutical

23 properties associated with retinoids, and are

24 particularly suitable for treatment of tumors, such

25 as acute monocytic leukemia, cervical carcinoma,

26 myeloma, ovarian carcinomas and head and neck

27 carcinomas, without having one or more undesirable

28 side effects of retinoids, such as inducement of

29 weight loss, mucocutaneous toxicity, skin irritation

30 and teratogenecity.

31   Accordingly, the present invention relates to

32 the use of RAR_α specific or selectiv retinoid

33 compounds for the treatment of dis as s and

34 conditions which respond to treatment by such

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1   compounds, and particularly to th tr atment of

2   tumors, primarily acute monocytic l ukemia, cervical

3   carcinoma, myeloma, ovarian carrcinomas and head and

4   neck carcinomas with the RAR$_\alpha$ specific or selective

5   retinoid compounds. In accordance with the present

6   invention the RAR$_\alpha$ selective compounds are also

7   particularly advantageously used for treatment of

8   proliferative vitreoretinopathy (PVR) and age

9   related macular degeneration (AMD).

10   For the purposes of the present description a

11 compound is considered RAR$_\alpha$ specific or selective if

12 in a transactivation assay (described below) the

13 compound transactivates the RAR$_\alpha$ receptors at a

14 significantly lower concentration than the RAR$_\beta$ and

15 RAR$_\Gamma$ receptors. Instead of measuring

16 transactivation, measuring the binding of a compound

17 respectively to the three RAR receptor subtypes is

18 also feasible. Binding data expressed in Kd numbers

19 obtained in a binding assay (described below) are

20 also indicative of a compound's ability to act

21 specifically or selectively on RAR$_\alpha$ receptors in

22 preference over RAR$_\beta$ and RAR$_\Gamma$ receptors. A compound

23 is considered RAR$_\alpha$ specific or selective for the

24 purposes of the present invention if the Kd number

25 for its binding to RAR$_\alpha$ receptors is approximately

26 500 times smaller than the Kd for its affinity to

27 RAR$_\beta$ and RAR$_\Gamma$ receptors.

28   **BRIEF DESCRIPTION OF THE DRAWING FIGURES**

29 **Figure 1** is a graph showing the results of an RPMI

30 8226 cell culture assay conducted with all trans

31 retinoic acid (ATRA) and two RAR$_\alpha$ selective compounds

32 in accordance with the present invention.

33   **Figure 2** is another graph showing the results of

34 an AML 193 cell culture assay conducted with two RAR$_\alpha$

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1   selective compounds in accordanc with the present

2   invention, and with two compounds which are not RAR$_\alpha$

3   selective.

4   **Figure 3** is still another graph showing results

5   of an AML 193 cell culture assay conducted with

6   three RAR$_\alpha$ selective compounds in accordance with the

7   present invention and with all trans retinoic acid

8   (ATRA).

9   **Figure 4** is a graph showing the proliferation of

10 ovarian tumor cells in a cell culture assay (EDR

11 assay) in the presence of varying concentrations of

12 **Compound 2** in accordance with the present invention.

13   **Figure 5** is a graph showing the RPE cell

14 proliferation in the presence of all trans retinoic

15 acid or **Compound 42** in the culture medium.

16   **Figure 6** is a graph showing the weight of a

17 group of experimental rats which were administered

18 for 3 days varying doses of an RAR$_\alpha$ selective

19 compound in accordance with the present invention.

20   **Figure 7** is a bar graph showing the weight of

21 a group of experimental rats at the end of a 4 day

22 period wherein for three days the rats were

23 administered varying doses of **Compound 18** in

24 accordance with the invention;

25   **Figure 8** is a graph showing the weight of guinea

26 pigs which were treated with varying doses of

27 **Compound 42** for 15 days.

28   **DETAILED DESCRIPTION OF THE INVENTIONGeneral**

29 **Embodiments**Definitions regarding the chemical

30 compounds used in the present invention

31   The term alkyl refers to and covers any and all

32 groups which are known as normal alkyl,

33 branched-chain alkyl and cycloalkyl. The term

34 alkenyl refers to and covers normal alkenyl, branch

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chain alkenyl and cycloalkenyl groups having one or
more sites of unsaturation. Similarly, the term
alkynyl refers to and covers normal alkynyl, and
branch chain alkynyl groups having one or more
triple bonds.

Lower alkyl means the above-defined broad
definition of alkyl groups having 1 to 6 carbons in
case of normal lower alkyl, and as applicable 3 to 6
carbons for lower branch chained and cycloalkyl
groups. Lower alkenyl is defined similarly having 2
to 6 carbons for normal lower alkenyl groups, and 3
to 6 carbons for branch chained and cyclo- lower
alkenyl groups. Lower alkynyl is also defined
similarly, having 2 to 6 carbons for normal lower
alkynyl groups, and 4 to 6 carbons for branch
chained lower alkynyl groups.

The term "ester" as used here refers to and
covers any compound falling within the definition of
that term as classically used in organic chemistry.
It includes organic and inorganic esters. Where **B**
in the general formula of the preferred compounds
used in the invention is -COOH, this term covers the
products derived from treatment of this function
with alcohols or thioalcohols preferably with
aliphatic alcohols having 1-6 carbons. Where the
ester is derived from compounds where **B** is $-CH_2OH$,
this term covers compounds derived from organic
acids capable of forming esters including
phosphorous based and sulfur based acids, or
compounds of the formula $-CH_2OCOR_{11}$ where R_{11} is any
substituted or unsubstituted aliphatic, aromatic,
heteroaromatic or aliphatic aromatic group,
pref rably with 1-6 carbons in the aliphatic
portions.

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1   Unless stat d otherwise in this application,

2   preferred esters are derived from the saturated

3   aliphatic alcohols or acids of ten or fewer carbon

4   atoms or the cyclic or saturated aliphatic cyclic

5   alcohols and acids of 5 to 10 carbon atoms.

6   Particularly preferred aliphatic esters are those

7   derived from lower alkyl acids and alcohols. Also

8   preferred are the phenyl or lower alkyl phenyl

9   esters.

10   Amides has the meaning classically accorded that

11   term in organic chemistry. In this instance it

12   includes the unsubstituted amides and all aliphatic

13   and aromatic mono- and di- substituted amides.

14   Unless stated otherwise in this application,

15   preferred amides are the mono- and di-substituted

16   amides derived from the saturated aliphatic radicals

17   of ten or fewer carbon atoms or the cyclic or

18   saturated aliphatic-cyclic radicals of 5 to 10

19   carbon atoms. Particularly preferred amides are

20   those derived from substituted and unsubstituted

21   lower alkyl amines. Also preferred are mono- and

22   disubstituted amides derived from the substituted

23   and unsubstituted phenyl or lower alkylphenyl

24   amines. Unsubstituted amides are also preferred.

25   Acetals and ketals include the radicals of the

26   formula-CK where K is $(-OR)_2$. Here, R is lower

27   alkyl. Also, K may be -OR,O- where R, is lower alkyl

28   of 2-5 carbon atoms, straight chain or branched.

29   A pharmaceutically acceptable salt may be

30   prepared for any compound used in this invention

31   having a functionality capable of forming such-salt,

32   for example an acid functionality. A

33   pharmac utically acceptable salt is any salt which

34   retains the activity of the parent compound and does

1   not impart any deleterious or untoward effect on the
2   subject to which it is administered and in the
3   context in which it is administered.
4   Pharmaceutically acceptable salts may be derived
5   from organic or inorganic bases. The salt may be a
6   mono or polyvalent ion. Of particular interest are
7   the inorganic ions, sodium, potassium, calcium, and
8   magnesium. Organic salts may by be made with
9   amines, particularly ammonium salts such as mono-,
10 di- and trialkyl amines or ethanol amines. Salts
11 may also be formed with caffeine, tromethamine and
12 similar molecules. Where there is a nitrogen
13 sufficiently basic as to be capable of forming acid
14 addition salts, such may be formed with any
15 inorganic or organic acids or alkylating agent such
16 as methyl iodide. Preferred salts are those formed
17 with inorganic acids such as hydrochloric acid,
18 sulfuric acid or phosphoric acid. Any of a number
19 of simple organic acids such as mono-, di- or tri-
20 acid may also be used.
21   Some of the compounds used in the present
22 invention may have trans and cis (E and Z) isomers.
23 In addition, the compounds used in the present
24 invention may contain one or more chiral centers and
25 therefore may exist in enantiomeric and
26 diastereomeric forms. The scope of the present
27 invention is intended to cover the use of all such
28 isomers per se, as well as mixtures of cis and trans
29 isomers, mixtures of diastereomers and racemic
30 mixtures of enantiomers (optical isomers) as well.
31 Description of the Compounds Preferably Used in the
32 Methods of the Invention
33   The retinoid-like compounds used in the methods
34 of treatment of the present invention are specific

12

1 or selective for RAR_α receptors. That a compound is

2 specific or selective to RAR_α receptors can be

3 ascertained in transactivation assays described

4 below where an RAR_α specific or selective compound

5 transactivates RAR_α receptors at a significantly

6 lower concentrations than RAR_β or RAR_γ receptors. In

7 a binding assay where the ability of the compound to

8 bind to these receptor subtypes is measured, a

9 compound that is considered RAR_α specific or

10 selective for the purposes of the present invention

11 binds at least approximately 500 times stronger to

12 RAR_α receptors than to the RAR_β or RAR_γ receptors.

13 Alternatively, the compound is considered RAR_α

14 specific or selective if in the binding assay its Kd

15 number is approximately in the 10^{-1} to 5×10^{2}

16 nanomolar range and the Kd number for RAR_β or RAR_γ

17 receptors is greater than 1000 nanmolar. The latter

18 is indicated by 0.00 in the below provided Tables

19 where binding data (Kd numbers) for certain

20 exemplary compounds of the present invention are

21 illustrated.

22   Examples for RAR_α selective compounds which are

23 preferably used in accordance with the present

24 invention are illustrated by Formula 1 and Formula 2

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33
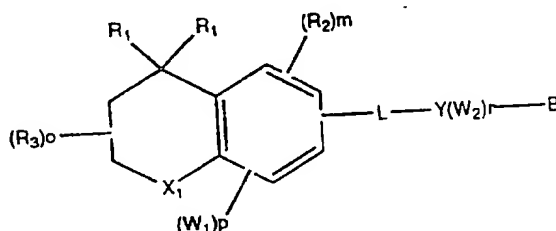
34   Formula 1   Formula 2

13
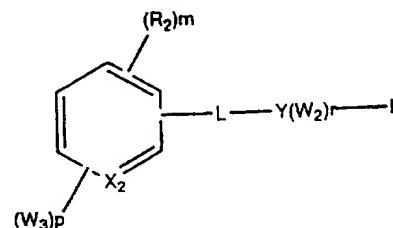
1 where X_1 is O or X_1 is $[C(R_1)_2]_n$ where n is an integer

2 between 0 and 2;

3   R_1 is independently H or alkyl of 1 to 6

4 carbons;

5   R_2 is independently hydrogen, or lower alkyl of

6 1 to 6 carbons;

7   R_3 is hydrogen, lower alkyl of 1 to 6 carbons or

8 F;

9   m is an integer having the value of 0 - 5;

10   o is an integer having the value of 0 - 4;

11   p is an integer having the value of 0 - 2;

12   r is an integer having the value 0 - 2;

13   X_2 is N or CH;

14   Y is a phenyl or naphthyl group, or heteroaryl

15 selected from a group consisting of pyridyl,

16 thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl,

17 thiazolyl, oxazolyl, imidazolyl and pyrrazolyl, said

18 phenyl, naphthyl and heteroaryl groups being

19 optionally substituted with one or two R_2 groups;

20   W_1 is a substituent selected independently from

21 the group consisting of F, Br, Cl, I, fluoro

22 substituted C_{1-6} alkyl, NO_2, and OH, with the provisos

23 that:

24   (i) when the compound is in accordance with

25 Formula 1 and Z is O then the sum of p and r is at

26 least 1 and W_1 is not a fluoro group in the 3

27 position of a tetrahydronaphthalene ring;

28   (ii) when the compound is in accordance with

29 Formula 1 and r is zero and p is 1 and W_1 is OH then

30 the OH group is positioned α to the L group;

31   W_2 is a substituent selected independently from

32 the group consisting of F, Br, Cl, I, fluoro

33 substituted C_{1-6} alkyl, NO_2, and OH;

34   W_3 is a substituent selected independently from

14

the group consisting of F, Br, Cl, I, C_{1-6}alkyl,

fluoro substituted C_{1-6} alkyl, NO_2, and OH with the

proviso that when the compound is in accordance with

Formula 2 and X_2 is CH and r is 0 then p is not 0 and

at least one W_3 group is not alkyl;

    L is -(C=Z)-NH- or -NH-(C=Z)-

    Z is O or S, and

    B is COOH or a pharmaceutically acceptable salt

thereof, $COOR_8$, $CONR_9R_{10}$, $-CH_2OH$, CH_2OR_{11}, CH_2OCOR_{11},

CHO, $CH(OR_{12})_2$, $CHOR_{13}O$, $-COR_7$, $CR_7(OR_{12})_2$, $CR_7OR_{13}O$,

where R_7 is an alkyl, cycloalkyl or alkenyl group

containing 1 to 5 carbons, R_8 is an alkyl group of 1

to 10 carbons or trimethylsilylalkyl where the alkyl

group has 1 to 10 carbons, or a cycloalkyl group of

5 to 10 carbons, or R_8 is phenyl or lower

alkylphenyl, R_9 and R_{10} independently are hydrogen,

an alkyl group of 1 to 10 carbons, or a cycloalkyl

group of 5-10 carbons, or phenyl or lower

alkylphenyl, R_{11} is lower alkyl, phenyl or lower

alkylphenyl, R_{12} is lower alkyl, and R_{13} is divalent

alkyl radical of 2-5 carbons.

    With reference to symbol X_1 in Formula 1,

compounds are preferred in the methods of the

present invention where X_1 is $[C(R_1)_2]_n$ and n is 1

(tetrahydronaphthalene derivatives) and also where X_1

is O (chroman derivatives). With reference to the

symbol X_2 in Formula 2, compounds are equally

preferred where X_2 is CH or N. When X_2 is CH then

the benzene ring is preferably 1, 3, 5 substituted

with the L group occupying the 1 position and the W_3

and/or R_2 groups occupying the 3 and 5 positions.

When the symbol X_2 is N, then the pyridine ring is

preferably 2,4,6 substituted with the L group

occupying the 4 position and the W_3 and/or R_2 groups

occupying the 2 and 6 positions.

The R_1 groups of F rmula 1 are preferably H or
CH_3. The R_3 group of Formula 1 is preferably H. The
group B of the preferred compounds of the invention
is COOH or a pharamceutically acceptable salt
thereof, $COOR_8$ or $CONR_9R_{10}$, where R_8, R_9 and R_{10} are
defined as above.

Referring now to the W_1 and W_2 groups in Formula
1, these groups are, generally speaking, electron
withdrawing groups, which are present in the
compounds of the invention either in the aromatic
portion of the condensed ring system, or as a
substituent of the aryl or heteroaryl group Y.
Preferably a W_2 group is present in the Y group, and
a W_1 group is also present in the aromatic portion of
the condensed ring system. When the Z group is S
(thioamides) a W_1 or W_2 group does not necessarily
have to be present in the compounds of the invention
in accordance with Formula 1, although preferably
at least one of the W_1 or W_2 groups is nevertheless
present. In the aryl or heteroaryl Y moiety in the
compounds of Formula 1 and Formula 2 as well, the W_2
group is preferably located in the position adjacent
to the B group; preferably the B group is in para
position in the phenyl ring relative to the "amide"
moiety, and therefore the W_2 group is preferably in
meta position relative to the amide moiety. Where
there is a W_1 group present in the aromatic portion
of the condensed ring system of the compounds of
Formula 1, it preferably occupies the 8 position of
the chroman nucleus with the Z=C-NH- group occupying
the 6 position. In tetrahydronaphthalene compounds
of Formula 1, the Z=C-NH- group is pr ferably in the
2-position, and the W_1 group is preferably in the 4

position. However, when the W_1 group is OH in
compounds of **Formula 1**, then the OH is preferably in
the 3 position of the tetrahydronaphthalene ring.

Preferred W_1 and W_2 groups are F, NO_2, Br, I,
CF_3, ClN_3, and OH. The presence of one or two
fluoro substituents in the Y group (W_2) is especially
preferred. When the Y group is phenyl, the fluoro
substituents preferably are in the _ortho_ and _ortho'_
positions relative to the B group, which is
preferably COOH or $COOR_4$.

Referring now to the W_3 group in **Formula 2**, this
group is, generally speaking, also an electron
withdrawing group or an alkyl group, more
specifically preferred W_3 groups are F, NO_2, Br, I,
CF_3, N_3, and OH. Alternatively, in the phenyl or
pyridyl ring (shown in **Formula 2** as substituent
"$(W_3)_p$") W_3 is an alkyl group, preferably
branch-chained alkyl, such as tertiary butyl, and
preferably p is 2.

With reference to the symbol Y in **Formula 1** and
in **Formula 2** as well, the preferred compounds used
in the methods of the invention are those where Y is
phenyl, pyridyl, 2-thiazolyl, thienyl, or furyl,
more preferably phenyl. As far as substitutions on
the Y (phenyl) and Y (pyridyl) groups are concerned,
compounds are preferred where the phenyl group is
1,4 (_para_) substituted by the L and B groups, and
where the pyridine ring is 2,5 substituted by the L
and B groups. (Substitution in the 2,5 positions in
the "pyridine" nomenclature corresponds to
substitution in the 6-position in the "nicotinic
acid" nomenclature.) In the pref rred compounds of
the invention there is no optional R_1 substituent
(other than H) on the Y group.

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1　　　　　Th　L group of Formula 1 and of Formula 2 is

2　preferably -(C=Z)-NH-, and Z is preferably O.　In

3　other words, those carbamoyl or amide compounds are

4　preferred in accordance with the present invention

5　where the -NH-moiety is attached to the Y group.

6　　　　　The compounds which are presently most

7　preferably used in the methods of treatment of the

8　invention are shown below in Table 1 with reference

9　to Formulas　3 and 4 and in Table 2 with reference

10　to　Formula 5.

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22　　　　　　　　　　　　Formula 3

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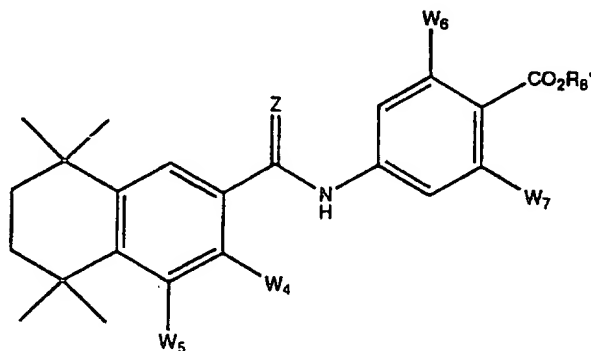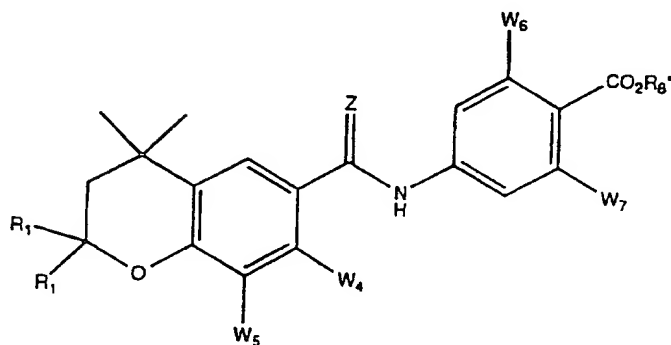
34　　　　　　　　　　　　Formula 4

Formula 5

TABLE 1

Compound

No.	Formula	$R_1°$	W_4	W_5	Z	W_6	W_7	R8°
1	3	--	H	H	O	F	H	Et
2	3	--	H	H	O	F	H	H
3	3	--	H	Br	O	F	H	Et
4	3	--	H	Br	O	F	H	H
5	3	--	OH	H	O	F	H	Et
6	3	--	OH	H	O	F	H	H
7	4	H	H	Br	O	F	H	Et
8	4	H	H	Br	O	F	H	H
9	4	CH_3	H	Br	O	F	H	Et
10	4	CH_3	H	Br	O	F	H	H
11	4	CH_3	H	CF_3	O	F	H	Et
12	4	CH_3	H	CF_3	O	F	H	H
13	4	CH_3	H	N_3	O	F	H	Et
14	4	CH_3	H	N_3	O	F	H	H
15	4	CH_3	H	CF_3	O	F	F	CH_3
16	4	CH_3	H	CF_3	O	F	F	H
17	4	CH_3	H	I	O	F	H	Et
18	4	CH_3	H	I	O	F	H	H
19	4	CH_3	H	CH_3	O	F	H	Et
20	4	CH_3	H	CH_3	O	F	H	H
21	3	--	H	H	S	H	H	Et
22	3	--	H	H	S	H	H	H
23	3	--	H	H	S	F	H	Et

24	3	--	H	H	S	F	H	H
25	3	--	H	Br	O	NO_2	H	CH_3
26	3	--	H	Br	O	NO_2	H	H
27	4	CH_3	H	H	O	F	H	Et
28	4	CH_3	H	H	O	F	H	H
29	3	--	OH	Br	O	F	H	Et
30	3	--	OH	Br	O	F	H	H
31	3	--	OH	Br	O	F	F	Me
32	3	--	OH	Br	O	F	F	H
33	3	--	H	H	O	F	F	Me
34	3	--	H	H	O	F	F	H

Table 2

Compound #	X_2	W_8	W_9	W_{10}	R°_8
41	N	H	F	H	Et
42	N	H	F	H	H
43	N	H	H	H	Et
44	N	H	H	H	H
45	CH	H	F	H	Et
46	CH	H	F	H	H
47	CH	OH	F	H	Et
48	CH	OH	F	H	H
49	N	H	F	F	Me
50	N	H	F	F	H
51	CH	H	F	F	Me
52	CH	H	F	F	H
53	N	H	NO_2	H	Me
54	N	H	NO_2	H	H

Modes of Administration

The RAR_α specific or selective compounds used in the methods of this invention may be administered systemically or topically, depending on such considerations as the condition to be treated, need

1   for site-specific treatment, quantity of drug to be
2   administered, and numerous other considerations.
3   In the treatment of dermatoses, it will
4   generally be preferred to administer the drug
5   topically, though in certain cases such as treatment
6   of severe cystic acne or psoriasis, oral
7   administration may also be used. Any common topical
8   formulation such as a solution, suspension, gel,
9   ointment, or salve and the like may be used.
10   Preparation of such topical formulations are well
11   described in the art of pharmaceutical formulations
12   as exemplified, for example, Remington's
13   Pharmaceutical Science, Edition 17, Mack Publishing
14   Company, Easton, Pennsylvania. For topical
15   application, these compounds could also be
16   administered as a powder or spray, particularly in
17   aerosol form. If the drug is to be administered
18   systemically, it may be confected as a powder, pill,
19   tablet or the like or as a syrup or elixir suitable
20   for oral administration. For intravenous or
21   intraperitoneal administration, the compound will be
22   prepared as a solution or suspension capable of
23   being administered by injection. In certain cases,
24   it may be useful to formulate these compounds by
25   injection. In certain cases, it may be useful to
26   formulate these compounds in suppository form or as
27   extended release formulation for deposit under the
28   skin or intramuscular injection.
29   Other medicaments can be added to such topical
30   formulation for such secondary purposes as treating
31   skin dryness; providing protection against light;
32   other medications for treating dermatoses;
33   medicaments for preventing infection, reducing
34   irritation, inflammation and the like.

1   Treatment of dermatoses or any other indications
2   known or discovered to be susceptible to treatment
3   by retinoic acid-like compounds will be effected by
4   administration of the therapeutically effective dose
5   of one or more compounds of the instant invention.
6   A therapeutic concentration will be that
7   concentration which effects reduction of the
8   particular condition, or retards it expansion. In
9   certain instances, the compound potentially may be
10   used in prophylactic manner to prevent onset of a
11   particular condition.
12   A useful therapeutic or prophylactic
13   concentration will vary from condition to condition
14   and in certain instances may vary with the severity
15   of the condition being treated and the patient's
16   susceptibility to treatment. Accordingly, no single
17   concentration will be uniformly useful, but will
18   require modification depending on the
19   particularities of the disease being treated. Such
20   concentrations can be arrived at through routine
21   experimentation. However, it is anticipated that in
22   the treatment of, for example, acne, or similar
23   dermatoses, that a formulation containing between
24   0.01 and 1.0 milligrams per mililiter of formulation
25   will constitute a therapeutically effective
26   concentration for total application. If
27   administered systemically, an amount between 0.01
28   and 5 mg per kg per day of body weight would be
29   expected to effect a therapeutic result in the
30   treatment of many disease for which these compounds
31   are useful.
32   In the treatment of tumors a dose of
33   approximately 0.5 to 5 mg per kg body weight per day
34   is anticipated to constitute the therapeutic dose.

1 Alternatively, as is performed fr quently in therapy

2 of malignancies, a patient is provided an initial

3 dose of 1 mg per kg body weight per day, and

4 therafter the dose is raised until a maximum

5 tolerated dose is attained.

6 <u>Assay of RAR$_\alpha$ receptor selective biological activity</u>

7 <u>and its significance in reduced side effects and</u>

8 <u>toxicity</u>

9   As it is noted in the introductory section of

10 this application for patent two main types of

11 retinoic acid receptors (RAR and RXR) exist in

12 mammals (and other organisms). Within each type

13 there are sub-types (RAR$_\alpha$, RAR$_\beta$, RAR$_\Gamma$, RXR$_\alpha$, RXR$_\beta$ and

14 RXR$_\Gamma$) the distribution of which is not uniform in the

15 various tissues and organs of mammalian organisms.

16 Selective binding of only one or two retinoid

17 receptor subtypes within one retinoid receptor

18 family can give rise to beneficial pharmacological

19 properties because of the varying distribution of

20 the sub-types in the several mammalian tissues or

21 organs. For the above-summarized reasons, binding

22 of any or all of the retinoid receptors, as well as

23 specific or selective activity in a receptor family,

24 or selective or specific activity in any one of the

25 receptor subtypes, are all considered desirable

26 pharmacological properties.

27   In light of the foregoing the prior art has

28 developed assay procedures for testing the agonist

29 like activity of compounds in the RAR$_\alpha$, RAR$_\beta$, RAR$_\Gamma$,

30 RXR$_\alpha$, RXR$_\beta$ and RXR$_\Gamma$ receptor subtypes. For example,

31 a chimeric receptor transactivation assay which

32 tests for agonist-like activity in the RAR$_\alpha$, RAR$_\beta$,

33 RAR$_\Gamma$, and RXR$_\alpha$ receptor subtyp s, and which is based

34 on work published by <u>Feigner P. L. and Holm M.</u>

1 (1989) Focus, 11 2 is described in detail in U.S.

2 Patent No. 5,455,265. The specification of United

3 States Patent No. 5,455,265 is expressly

4 incorporated herein by reference.

5   A holoreceptor transactivation assay and a

6 ligand binding assay which measure the ability of

7 compounds to bind to the several retinoid receptor

8 subtypes, respectively, are described in published

9 PCT Application No. WO WO93/11755 (particularly on

10 pages 30 - 33 and 37 - 41) published on June 24,

11 1993, the specification of which is also

12 incorporated herein by reference. A description of

13 the ligand binding assay is also provided below.

14 BINDING ASSAY

15   All binding assays were performed in a similar

16 fashion. All six receptor types were derived from

17 the expressed receptor type (RAR α, β, Γ and RXR α,

18 β, Γ) expressed in Baculovirus. Stock solutions of

19 all compounds were prepared as 10mM ethanol

20 solutions and serial dilutions carried out into 1:1

21 DMSO; ethanol. Assay buffers consisted of the

22 following for all six receptor assays: 8% glycerol,

23 120mM KCl, 8mM Tris, 5mM CHAPS 4mM DTT and 0.24mM

24 PMSF, pH - 7.4@ room temperature.

25   All receptor binding assays were performed in

26 the same manner. The final assay volume was 250μl

27 and contained from 10-40μg of extract protein

28 depending on receptor being assayed along with 5 nM

29 of [^{3}H] all-trans retinoic acid or 10nM [^{3}H] 9-cis

30 retinoic acid and varying concentrations of

31 competing ligand at concentrations that ranged from

32 0 - 10^{-5} M. The assays w re formatted for a 96 well

33 minitube system. Incubations were carried out at

34 4°C until equilibrium was achieved. Non-specific

24

binding was defined as that binding r maining in the

presence of 1000nM of the appropriate unlabeled

retinoic acid isomer. At the end of the incubation

period, 50μl of 6.25% hydroxyapitite was added in

the appropriate wash buffer. The wash buffer

consisted of 100mM KCl, 10mM Tris and either 5mM

CHAPS (RXR α, β, Γ) or 0.5% Triton X-100 (RAR α, β,

Γ). The mixture was vortexed and incubated for 10

minutes at 4°C, centrifuged and the supernatant

removed. The hydroxyapitite was washed three more

times with the appropriate wash buffer. The

receptor-ligand complex was adsorbed by the

hydroxyapitite. The amount of receptor-ligand

complex was determined by liquid scintillation

counting of hydroxyapitite pellet.

   After correcting for non-specific binding, IC_{50}

values were determined. The IC_{50} value is defined as

the concentration of competing ligand needed to

reduce specific binding by 50%. The IC_{50} value was

determined graphically from a loglogit plot of the

data. The K_d values were determined by application

of the Cheng-Prussof equation to the IC_{50} values, the

labeled ligand concentration and the K_d of the

labeled ligand.

   The results of **ligand binding assay** are expressed

in K_d numbers. (See <u>Cheng et al.</u> Biochemical

Pharmacology Vol. 22 pp 3099-3108, expressly

incorporated herein by reference.)

   Table 3 shows the results of the ligand binding

assay for certain exemplary compounds of the

invention.

25

TABLE 3

Ligand Binding Assay

Compound #	K_d (nanomolar)					
	RARα	RARß	RARΓ	RXRα	RXRß	RXRΓ
2	1.90	480.0	0.00	0.00	0.00	0.00
4	1.3	0.00	0.00	0.00	0.00	0.00
6	3.00	0.00	0.00	0.00	0.00	0.00
10	24.0	0.00	0.00	0.00	0.00	0.00
12	14.0	0.00	0.00	0.00	0.00	0.00
14	52.0	0.00	0.00	0.00	0.00	0.00
16	51.0	0.00	0.00	0.00	0.00	0.00
18	16.0	0.00	0.00	0.00	0.00	0.00
20	57.0	0.00	0.00	0.00	0.00	0.00
22	15	0.00	0.00	0.00	0.00	0.00
24	7.5	0.00	0.00	0.00	0.00	0.00
26	245.0	0.00	0.00	0.00	0.00	0.00
28	162.0	0.00	0.00	0.00	0.00	0.00
30	<3.00	0.00	0.00	0.00	0.00	0.00
32	2.30	0.00	0.00	0.00	0.00	0.00
34	9.00	0.00	0.00	0.00	0.00	0.00
42	14.00	0.00	0.00	0.00	0.00	0.00
44	19.00	0.00	0.00	0.00	0.00	0.00
46	26.0	0.00	0.00	0.00	0.00	0.00
48	77.0	0.00	0.00	0.00	0.00	0.00
50	62.0	0.00	0.00	0.00	0.00	0.00
52	87.0	0.00	0.00	0.00	0.00	0.00
54	94.0	0.00	0.00	0.00	0.00	0.00
TTNPB[1]	72	5	36			

0.00 indicates value greater than 1000nM (nanomolar)

[1] TTNPB is a well known prior art retinoid (4-(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)propen-1-yl)benzoic acid, that is not RARα selective.

26

As it can be seen from the foregoing data, the
compounds used in accordance with the present
invention specifically or selectively bind to RAR$_\alpha$
retinoid receptors. It has been discovered in
accordance with the present invention that this
unique type of selectivity allows the compounds to
retain beneficial retinoid-like properties while
reduces certain side effects and toxicity. More
specifically, certain _in vitro_ cell culture assays
are described below, in which the ability of the RAR$_\alpha$
specific or selective compounds to significantly
inhibit the growth of cancer cells is demonstrated.

CANCER CELL LINE ASSAYS

MATERIALS AND METHODS

Hormones

All trans-retinoic acid (t-RA) (Sigma Chemicals
Co., St. Louis, MO) was stored at -70°C. Prior to
each experiment the compound was dissolved in 100%
ethanol at 1 mM and diluted in culture medium
immediately before use. All experiments were
performed in subdued light. Controls were assayed
using the same concentration of ethanol as present
in the experimental plates and this concentration of
diluent had no effect in either assay.

Cells and Cell Culture

The cell lines, RPMI 8226, ME-180 and AML-193
were obtained from the American Type Culture
Collection (ATCC, Rockville, MD). RPMI 8226 is a
human hematopoietic cell line obtained from the
peripheral blood of a patient with multiple myeloma.
The cells resemble the lymphoblastoid cells of other
human lymphocyte cell lines and secrete α-type light
chains of immunoglobulin. RPMI-8226 cells are grown
in RPMI medium (Gibco) supplemented with 10% fetal

1 bovine serum, glutamine and antibiotics. Th cells

2 were maintained as suspension cultures grown at 37°C

3 in a humidified atmosphere of 5% CO_2 in air. The

4 cells were diluted to a concentration of 1 x 10^5/ml

5 twice a week.

6 ME-180 is a human epidermoid carcinoma cell line

7 derived from the cervix. The tumor was a highly

8 invasive squamous cell carcinoma with irregular cell

9 clusters and no significant keratinization. ME-180

10 cells were grown and maintained in McCoy's 5a medium

11 (Gibco) supplemented with 10% fetal bovine serum,

12 glutamine and antibiotics. The cells were

13 maintained as monolayer cultures grown at 37°C in a

14 humidified atmosphere of 5% CO_2 in air. The cells

15 were diluted to a concentration of 1 x 10^5/ml twice a

16 week.

17 AML-193 was established from the blast cells

18 classified as M5 Acute Monocyte Leukemia. The

19 growth factor, granulocyte colony-stimulation factor

20 (GM-CSF) was required to establish this cell line

21 and growth factors are necessary for its continuous

22 proliferation in chemically defined medium. AML-193

23 cells were grown and maintained in Iscove's modified

24 Dulbecco's medium supplemented with 10% fetal bovine

25 serum, glutamine and antibiotics with 5µg/ml insulin

26 (Sigma Chemical Co.) and 2 ng/ml rh GM-CSF (R and D

27 Systems). The cells were diluted to a concentration

28 of 3 x 10^5/ml twice a week.

29 Incorporation of ^{3}H-Thymidine

30 The method used for determination of the

31 incorporation of radiolabeled thymidine was adapted

32 from the procedure described by Shrivastav et al.

33 RPMI-8226 cells were plated in a 96 well round

34 bottom microtiter plate (Costar) at a density of

1 1,000 cells/w 11. To appropriat wells, retinoid
2 test compounds were added at the final
3 concentrations indicated for a final volume of 150
4 µl/well. The plates were incubated for 96 hours at
5 37°C in a humidified atmosphere of 5% CO_2 in air.
6 Subsequently, 1 µCi of [5'-^{3}H]-thymidine (Amersham,
7 U.K. 43 Ci/mmol specific activity) in 25 µl culture
8 medium was added to each well and the cells were
9 incubated for an additional 6 hours. The cultures
10 were further processed as described below.

11   ME-180 wells, harvested by trypsinization were
12 plated in a 96 well flat bottom microtiter plate
13 (Costar) at a density of 2,000 cells/well. The
14 cultures were treated as described above for RPMI
15 8226 with the following exceptions. After
16 incubation with thymidine the supernatant was
17 carefully removed, and the cells were washed with a
18 0.5 mM solution of thymidine in phosphate buffered
19 saline. ME180 cells were briefly treated with 50µl
20 of 2.5% trypsin to dislodge the cells from the
21 plate.

22   AML-193 cells were plated in a 96 well round
23 bottom microtiter plate (Costar) at a density of
24 1,000 cells/well. To appropriate wells, retinoid
25 test compounds were added at the final
26 concentrations indicated for a final volume of 150
27 µl/well. The plates were incubated for 96 hours at
28 37°C in a humidified atmosphere of 5% CO_2 in air.
29 Subsequently, 1 µCi of [5'-^{3}H]-thymidine (Amersham,
30 U.K., 43 Ci/mmol specific activity) in 25 µl culture
31 medium was added to each well and the cells were
32 incubated for an additional 6 hours.

33   The cell lines were then processed as follows:
34 the cellular DNA was precipitated with 10%

29

1   trichloroacetic acid onto glass fiber filter mats

2   using a SKATRON multi-well cell harvester (Skatron

3   Instruments, Sterling VA). Radioactivity

4   incorporated into DNA, as a direct measurement of

5   cell growth, was measured by liquid scintillation

6   counting. The numbers represent the mean

7   disintegrations per minute of incorporated thymidine

8   from triplicate wells ± SEM.

9   The graph of Figure 1 of the appended drawings

10 shows that in the above described RPMI 8226 cell

11 (malignant myeloma) culture assay Compounds 4 and 12

12 (two exemplary compounds used in accordance with

13 this invention) inhibited the growth of these

14 malignant cells, substantially as well as a

15 comparison compound, all trans retinoic acid (ATRA).

16 The graph of Figure 1 also demonstrates that whereas

17 in a low concentration range (10^{-12} to approximately

18 10^{-9}) all trans retinoic acid (ATRA) actually

19 facilitates growth of these cells, the RAR_α selective

20 Compounds 4 and 12 of the present invention do not

21 stimulate but rather already in this low

22 concentrations inhibit the growth of these malignant

23 cells.

24   The graph of Figure 2 shows that in the above

25 described AML 193 (acute monocytic leukemia) cell

26 culture assay Compounds 22 and 42 in accordance with

27 this invention inhibited the growth of these

28 malignant cells. Two other compounds for which data

29 are also shown in this graph are designated AGN

30 193090 and AGN 193459. (An AGN number is an

31 arbitrary designation number used by the corporate

32 assigne of the present invention.) The compounds

33 AGN 193090 and AGN 193459 are not RAR_α selective.

34 These compounds respectively are

30

1 4-[(8-cyano-5,6-dihydro-5,5-dimethylnaphth-2-yl)ethy

2 nyl]benzoic acid, and

3 4-[(5,6-dihydro-5,5-dimethylnaphth-7(6H)-8-(1-2,2-di

4 methylpropylidene)naphth-2-yl)ethynyl]benzoic acid,

5 and their Kd values for RAR_α, RAR_β and RAR_Γ receptors

6 are 109, 34, 77 and 6, 2, 7, respectively. The

7 graph of Figure 2 demonstrates that the RAR_α

8 selective or specific compounds inhibit the

9 malignant cell growth at low concentrations where

10 the pan agonist AGN 193090 and AGN 193459 compounds

11 do not inhibit but rather at these low

12 concentrations even stimulate such cell growth.

13   Figure 3 is another graph showing the results of

14 an AML-193 cell culture assay, where Compounds 4, 12

15 and 18 in accordance with the present invention, and

16 all trans retinoic acid (ATRA) were tested. The

17 data show that the RAR_α selective compounds reduce

18 cell proliferation at low concentrations whereas

19 ATRA at the same low concentration actually promotes

20 cell proliferation.

21   In another line of assays the effect of the

22 retinoid compounds is tested against cells obtained

23 from solid tumors of patients. This EDR assay is

24 described below as follows:

25   Freshly resected solid tumor biopsies were

26 received within 24 hours of surgery. Species were

27 processed for assay after retaining a portion of the

28 tumor for paraffin embedding and histopathologic

29 confirmation of specimen viability and tissue

30 diagnosis. The remaining specimen was dissociated

31 into small fragments using sterile scissors. The

32 small tissue fragments were then exposed to

33 collagenase and DNAase for 2 hours with mixing a CO_2

34 incubator in order to release the tumor cells from

1   the connectiv tissue stroma. The resulting cell
2   suspension was washed, and c 11 counts determined
3   from a cytospin preparation. Tumor cells were
4   resuspended at 40,000 cells per ml in 0.3% agarose
5   in RMPI 1640 supplemented with 15% FCS, glutamine
6   and antibiotics, and 0.5 ml were plated into each
7   well of a 24 well plate over 0.5 ml layer of 0.5%
8   agarose. These culture conditions prevent cell
9   adherence, thereby allowing only transformed cells
10 to proliferate. Additionally, the cells grow into
11 three dimensional spheroids, recapitulating their in
12 vivo morphology.
13   Retinoid drugs were added 24 hours after plating
14 to insure specimen reequilibration to a growth
15 environment after the rigors of transport and
16 processing. Cells were grown for four days in the
17 presence of drug, with ^{3}H-thymidine (5 uCi/ml) added
18 48 hours prior to harvest to insure adequate
19 labeling of proliferating cells. After the
20 agarose-cell suspension was liquefied at 90°C, cells
21 were harvested onto glass fiber filters, which were
22 counted in 5 ml scintillation fluid using a Beckman
23 6500 liquid scintillation counter.
24   Results are reported as fraction of untreated
25 control cell proliferation. Treatment groups were
26 performed in duplicate or triplicate, while the
27 controls were performed in quadruplicate.
28   The graph of Figure 4 shows the effect of
29 Compound 2 on ovarian tumors obtained from 4
30 patients, and demonstrates that the compound
31 inhibits this tumor cell proliferation in a
32 concentration dependent manner.
33   It will be understood by those skilled in the
34 art, that the ability of the RAR_α selective compounds

32

1   to significantly inhibit growth of malignant cells

2   in the above described assays is an indication that

3   these compounds can be administered with beneficial

4   effect to tumor bearing mammals (including humans)

5   for the treatment of tumors, particularly acute

6   monocytic leukemia, cervical carcinoma, myeloma,

7   ovarian carcinomas and head and neck carcinomas.

8   It has also been discovered in accordance with

9   the present invention that the proliferation of

10 retinal pigment epithelium cells is inhibited by RAR_α

11 selective compounds. By way of background it is

12 noted that after retinal detachment the retinal

13 pigment epithelium (RPE) becomes dedifferentiated,

14 proliferates and migrates into the subretinal space

15 (Campochiaro et al., Invest. Opthal & Vis. Sci.

16 32:65-72 (1991)). Such processes therefore have an

17 impact upon the success of retinal reattachment

18 procedures. RAR agonists such as all-trans-retinoic

19 acid (ATRA) exhibit an antiproliferative effect upon

20 the growth rate of primary human RPE cultures

21 (Campochiaro et al., ibid) and have been shown to

22 decrease the incidence of retinal detachment after

23 retinal reattachment surgery in human studies

24 (Fekrat et al., Opthamology 102:412-418 (1994)).

25   The graph of Figure 5 shows the concentration

26 dependent inhibitory effect of all trans retinoic

27 acid (ATRA) and of Compound 42 on RPE proliferation

28 in an assay procedure which is described below.

29 Analysis of primary RPE cultures

30   Primary cultures of human retinal pigment

31 epithelium (RPE) were established from eyes as

32 previously described, (Campochiaro et al., Invest.

33 Opthal & Vis. Sci. 32:65-72 (1991)). 5 X 10^4 Cells

34 were plated in 16-mm wells of 24-well multiwell

33

1   plates in Dulb cco's modified Eagle's medium (DMEM

2   Gibco) containing 10% fetal bovine serum (FBS).

3   Cells were treated with ethanol alone (control),

4   ATRA (10^{-10} to 10^{-6} M) in ethanol, and Compound 42

5   (10^{-10} to 10^{-6} M) in ethanol. Cells were fed with

6   fresh media containing the appropriate

7   concentrations of these compounds every two days for

8   a total of six days treatment. Cells were removed

9   from the plates via treatment with trypsin and the

10 number of cells were counted with an electronic cell

11 counter. As it can be seen in Figure 5 treatment of

12 primary RPE cells with ATRA and with Compound 42

13 both led to a dose dependent decrease in RPE cell

14 proliferation.

15   The effect of topically administering to

16 experimental hairless mice RAR_α selective retinoid

17 compounds in accordance with the present invention

18 was also evaluated in a topical skin irritation

19 assay, using the RAR_α selective Compound 18 of the

20 invention. More particularly, skin irritation was

21 measured on a semi-quantitative scale by the daily

22 subjective evaluation of skin flaking and abrasions.

23 A single number, the topical irritation score,

24 summarizes the skin irritation induced in an animal

25 during the course of an experiment. The topical

26 irritation score is calculated as follows. The

27 topical irritation score is the algebraic sum of a

28 composite flaking score and a composite abrasion

29 score. The composite scores range from 0-9 and 0-8

30 for flaking and abrasions, respectively, and take

31 into account the maximum severity, the time of

32 onset, and the average severity of the flaking and

33 abrasions observed.

34   The severity of flaking is scored on a 5-point

34

scale and the severity of abrasions is scored on a
4-point scale, with higher scores reflecting greater
severity. The maximum severity component of the
composite scores would be the highest daily severity
score assigned to a given animal during the course
of observation.

For the time of onset component of the composite
score, a score ranging from 0 to 4 is assigned as
follows:

Time to Appearance of
Flaking or Abrasions of
Severity 2 or greater
            (days)   Time of Onset Score
      _____   _____
            8   0
           6-7   1
            5   2
           3-4   3
           1-2   4

The average severity component of the composite
score is the sum of the daily flaking or abrasion
scores divided by the number of observation days.
The first day of treatment is not counted, since the
drug compound has not had an opportunity to take
effect at the time of first treatment.
To calculate the composite flaking and abrasion
scores, the average severity and time of onset
scores are summed and divided by 2. The result is
added to the maximal severity score. The composite
flaking and abrasion scores are then summed to give
the overall topical irritation score. Each animal
receives a topical irritation score, and the values

are expressed as the mean ± SD of the individual

scores of a group of animals. Values are rounded to

the nearest integer.

   Thus, female hairless mice [Crl:SKH1-hrBR] (8-12

weeks old, n=4) were treated topically for 5

consecutive days with Compound 18 in doses expresed

in nanomol/25 g, which is particularly given in

Table 4. Treatments are applied to the dorsal skin

in a total volume of 4 ml/kg (~0.1 ml). Mice were

observed daily and scored for flaking and abrasions

up to and including 3 days after the last treatment,

i.e., day 8.

Table 4
Eight Day Topical Assay in Hairless Mice
of Compound 18

Dose	Mortality (out of 4)	Body Weight % gain or (loss)	Flaking Score	Abrasion Score	Composite Score
100	0	8 ± 7	0	1	1 ± 1
1000	0	4 ± 1	1	1	2 ± 0
of TTNPB					
0.9	0	5 ± 2	5	3	8 ± 2
2.7	0	(4 ± 3)	6	3	9 ± 2
9	0	(11 ± 3)	7	5	11 ± 2

   These data show that the RAR_α selective compound

causes virtually no skin irritation and no weight

36

1   loss up to 1000 nmol/25g in the t st model. For
2   comparison it should be noted that the well known
3   prior art retinoid compound
4   4-(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnapht
5   halen-2-yl)propen-1-yl)benzoic acid (TTNPB), which
6   is not RAR_α selective, causes much more serious skin
7   irritation in the above-noted test, as is shown in
8   the foregoing table.
9   Another important advantage of administering
10 RAR_α selective retinoid compounds to a mammal lies in
11 the significantly reduced teratogenic potency of the
12 RAR_α selective compounds compared to many other
13 retinoids, as measured by a chondrogenesis
14 suppression bioassay. This assay is performed as
15 follows:
16   High-density "spot" cultures of limb bud
17 mesenchymal cells are used to compare the ability of
18 various concentrations of test drugs to suppress
19 chondrogenic differentiation as a bioassay.
20 Forelimb buds of mouse embryos on day 12 of
21 gestation (54 ± 2 somites) are dissociated in a
22 trypsin-EDTA solution, and the resultant single-cell
23 suspension is plated as 20-μl spots (200,000
24 cells/spot) on plastic culture dishes. Retinoid
25 concentrations ranging from 0.3 ng/ml to 3 μg/ml (1
26 nM-10 μM) are added to the culture medium (Eagle's
27 MEM + 10% fetal bovine serum, GIBCO) 24 hours after
28 initial plating. Control cultures receive only the
29 vehicle (ethanol, concentration ≤ 1% by vol);
30 Retinoic acid is used as a positive control in
31 another set of cultures.
32   The cultures are terminated 96 hours after
33 plating, at which time the medium is r moved and the
34 cells are fixed for 1 hour in 10% formalin

37

1   containing 0.5% c tylpyridinium chloride. The

2   cultures are rinsed in acetic acid and stained for 1

3   hour in 0.5% Alcian blue solution at pH 1.0,

4   differentiated in 3% acetic acid, and then

5   dehydrated in ethanol and scored for chondrogenesis

6   under the microscope. An absence or reduction in

7   the number of cartilage nodules in stained cultures

8   as compared with control cultures is taken as a

9   measure of suppression of chondrogenesis. The

10   number of cartilage nodules stained in the whole

11   spot, mean number of nodules, and standard

12   deviations are calculated for four replicate

13   cultures per treatment. The median concentration

14   causing a 50% inhibition of chondrogenesis compared

15   with controls (IC_{50}) is calculated by logarithmic

16   curve fitting of the dose-response data. The IC_{50}

17   values are expressed in nanogram per mililiter

18   (ng/ml) units. An IC_{50} value of greater

19   concentration in this assay signifies lesser

20   teratogenecity. Table 5 indicates the results

21   obtained in this assay for Compounds 10, 18, and 42

22   in accordance with the present invention, as well as

23   for comparison with all trans retinoic acid (ATRA)

24   and 4-(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetra-

25   methylnaphtha-len-2-yl)propen-1-yl)benzoic acid

26   (TTNPB).

27

28   Table 5

Compound	IC_{50} (ng/ml)
10	250
18	220
42	65
ATRA	55
TTNPB	0.01

38

As it can be seen the compounds used in
accordance with the present invention are less
teratogenic than all trans retinoic acid and
significantly (of the 10^4 order of magnitude) less
teratogenic than the prior art TTNPB compound.

Weight loss or gain that experimental animals
experience upon administration of retinoid compounds
is another test of the drug's toxicity, with
significant weight loss at relatively low doses
indicating a significant toxic side effect of the
retinoid. In one experiment, groups of 5 rats were
treated with varying doses (administered in corn
oil) of a test retinoid for 3 days. The rats were
euthanized 24 hours after the last dose. The graph
of Figure 6 shows the average weight of each group
of rats treated with a daily dose of 10, 30, and 90
μmol/kg/day of Compound 42, as well as the average
weight of a group of control rats which were not
given the retinoid. As it can be seen, the RAR_α
selective Compound 42 caused virtually no weight
loss, as compared to the control, except in a very
high dose (90 μmol/kg/day). The graph of Figure 7
shows the weight of the rats on the fourth day (24
hours after last administration of retinoid) in a
similar test with varying doses of Compound 18, with
a zero dose indicating the control. As it can be
seen, this RAR_α selective retinoid caused virtually
no weight loss even in the high dose of 90
μmol/kg/day. It is noteworthy that in similar tests
TTNPB, which binds to all three RAR receptor
subtypes (see Table 3) causes very significant
weight loss. In this experim nt involving the rats
treated with Compound 42, significant mucocutaneous
toxicity was not observed.

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1   In another xperiment three-week old male
2   Hartley guinea pigs were implanted intraperitonially
3   with osmotic pumps containing 20 % DMSO/80
4   polyethylene glycol (vehicle) or Compound 42 at
5   concentrations of 4.4, 13.3 or 40 mg/ml in vehicle.
6   Based on the initial body weights and known pumping
7   rate, approximate doses of 0, 2, 6, and 18 mg/kg/day
8   doses of Compound 42 are estimated. Body weights
9   and clinical observations were recorded at least
10   every other day for 14 days post-implantation. The
11   guinea pigs were euthanized after 14 days, and the
12   pumps were examined for possible failure. The graph
13   of Figure 8 shows the weight of the animals involved
14   in this experiment over the course of 15 days. As
15   it can be seen from the graph, the lower and middle
16   doses of the RAR_α selective retinoid compound
17   (Compound 42) caused no, or only statistically
18   insignificant depression of weight gain, relative to
19   the control animals. Significant depression of
20   weight gain was observed only in the high dose
21   (18mg/kg/day) of Compound 42. Importantly, no signs
22   of mucocutaneous toxicity were observed at any dose
23   of Compound 42 in this experiment. The foregoing,
24   markedly reduced mucocutaneous toxicity observed
25   when animals are treated with RAR_α selective
26   compounds in accordance with the present invention,
27   is a significant advantage, because mucocutaneous
28   toxicity is the major and most irksome retinoid side
29   effect or toxicity in human patients.
30   <u>Synthetic Methods for Preparing the Preferred</u>
31   <u>Examples of RAR_α Selective Compounds of the Invention</u>
32   General structure of the compounds which are
33   preferably used in the m thods of treatment of the
34   pr sent invention are shown above in Formula 1 and

1   **Formula 2.** These compounds can be made by the

2   synthetic chemical pathways illustrated here. The

3   synthetic chemist will readily appreciate that the

4   conditions set out here are specific embodiments

5   which can be generalized to any and all of the

6   compounds represented by these formulas.

7   Generally speaking the process of preparing

8   compounds preferably used in the methods of the

9   invention in accordance with **Formula 1** involves the

10 formation of an amide by the reaction of a compound

11 of the general **Formula 6** with a compound of general

12 **Formula 7**, or by the reaction of a compound of

13 general **Formula 6a** with a compound of general

14 **Formula 7a.** Similarly, the process of preparing

15 compounds in accordance with **Formula 2** involves the

16 formation of an amide by the reaction of a compound

17 of the general **Formula 8** with a compound of general

18 **Formula 7**, or by the reaction of a compound of

19 general **Formula 8a** with a compound of general

20 **Formula 7a.**

21   A compound of **Formula 6** is an acid or an

22 "activated form" of a carboxylic acid attached to

23 the aromatic portion of a tetrahydronaphthalene, (X_1

24 = $[C(R_1)_2]_n$ and n is 1), dihydroindene ($[C(R_1)_2]_n$ where

25 n is 0) or chroman (X_1 is O) nucleus. The carboxylic

26 acid, or its "activated form" is attached to the 2

27 or 3 position of the tetrahydronaphthalene, and to

28 the 6 or 7 position of the chroman moieties. In the

29 compounds preferably used in accordance with the

30 invention the attachment is to the 2 position of

31 tetrahydronaphthalene and to the 6 position of

32 chroman.

33   The term "activat d form" of the carboxylic acid

34 should be understood in this regard as such

1   derivativ of the carboxylic acid which is capable

2   of forming an amide wh n reacted with a primary

3   amine of Formula 7. In case of the "reverse amides"

4   the activated form of a carboxylic acid is a

5   derivative (Formula 7a) that is capable of forming

6   an amide when reacted with a primary amine of

7   Formula 6a. This, generally speaking, means such

8   derivatives of a carboxylic acid which are normally

9   known and used in the art to form amide linkages

10 with an amine. Examples of suitable forms or

11 derivatives for this purpose are acid chlorides,

12 acid bromides, and esters of the carboxylic acid,

13 particularly active esters, where the alcohol moiety

14 of the ester forms a good leaving group. Presently

15 most preferred as reagents in accordance with

16 Formula 6 (or Formula 7a) are acid chlorides (X_3 is

17 Cl). The acid chlorides of Formula 6 (or of Formula

18 7a) can be prepared by traditional methods from the

19 corresponding esters (X_3 is for example ethyl) by

20 hydrolysis and treatment with thionyl chloride

21 (SO_2Cl). The acid chlorides of Formula 6 (or of

22 Formula 7a) can also be prepared by direct treatment

23 of the carboxylic acids with thionyl chloride, where

24 the carboxylic acid, rather than an ester thereof is

25 available commercially or by a known synthetic

26 procedure. The acid chlorides of Formula 6 (or of

27 Formula 7a) are typically reacted with the amine of

28 Formula 7 (or amine of Formula 6a) in an inert

29 solvent, such as methylene chloride, in the presence

30 of an acid acceptor, such as pyridine.

31   The carboxylic acids themselves in accordance

32 with Formula 6 (or Formula 7a) are also suitable for

33 amide formation when reacted with an amine, a

34 catalyst (4-dimethylaminopyridine) in the presence

42

1   of a dehydrating agent, such as

2   dicyclohexylcarbodiimide (DCC) or more preferably

3   1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

4   hydrochloride (EDC).

5   The carboxylic acids or the corresponding esters

6   of Formula 6, are generally speaking, prepared as

7   described in the chemical scientific or patent

8   literature and the literature procedures for their

9   preparation may be modified, if necessary, by such

10 chemical reactions or processes which per se are

11 known in the art. For example, generally speaking,

12 2,2, 4,4 and/or 2,2,4,4-substituted chroman

13 6-carboxylic acids and chroman 7-carboxylic acids

14 are available in accordance with the teachings of

15 United States Patent Nos. 5,006,550, 5,314,159,

16 5,324,744, and 5,348,975, the specifications of

17 which are expressly incorporated herein by

18 reference. 5,6,7,8-Tetrahydronaphthalene-2-

19 carboxylic acids are, generally speaking, available

20 in accordance with the teachings of United States

21 Patent No. 5,130,335, the specifications of which is

22 expressly incorporated herein by reference.

23   The foregoing general description of the

24 reactions which lead to formation of the amides of

25 Formula 1 is also, generally speaking, applicable to

26 the formation of the amides of Formula 2. The

27 reagents which are used in accordance with the

28 general principles mentioned above for the formation

29 of amide compounds of Formua 2 are: activated forms

30 of a carboxylic acids shown in Formula 8 and in

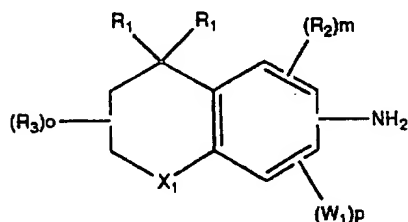31 Formula 7a, and the amines of Formula 7 and of

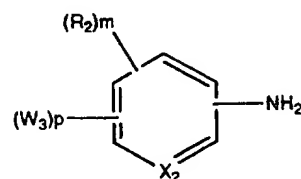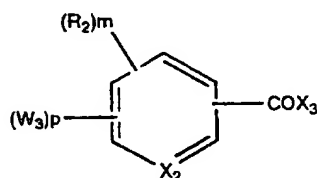32 Formula 8a.

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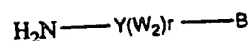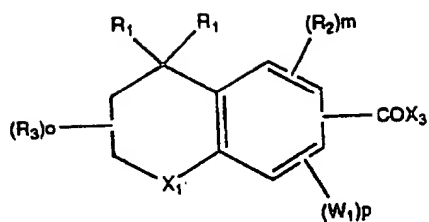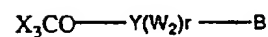
Formula 6


Formula 7

Formula 6a
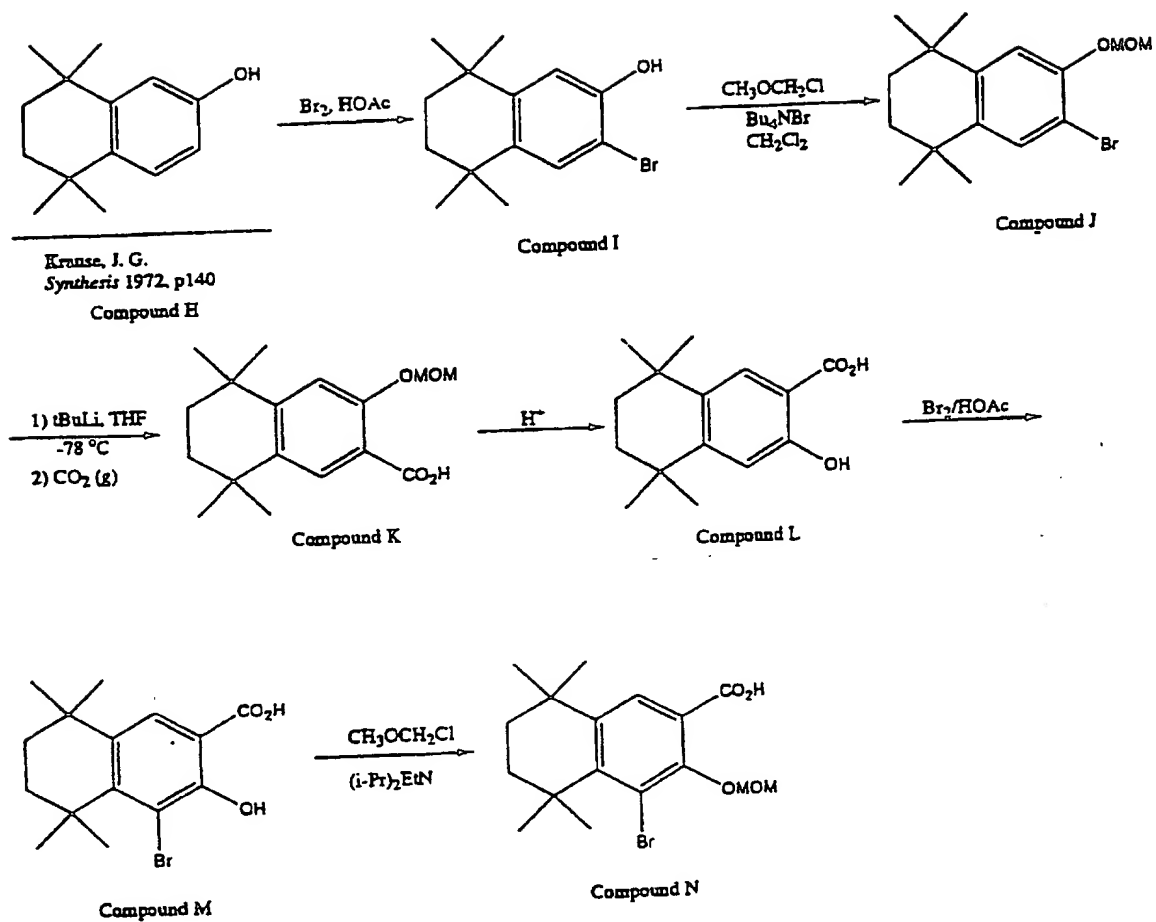

Formula 7a

Formula 8

Formula 8a

The carboxylic acids or the corresponding esters
of Formula 8, are generally speaking, prepared as
described in the chemical scientific or patent
literature and the literature procedures for their
preparation may be modified, if necessary, by such
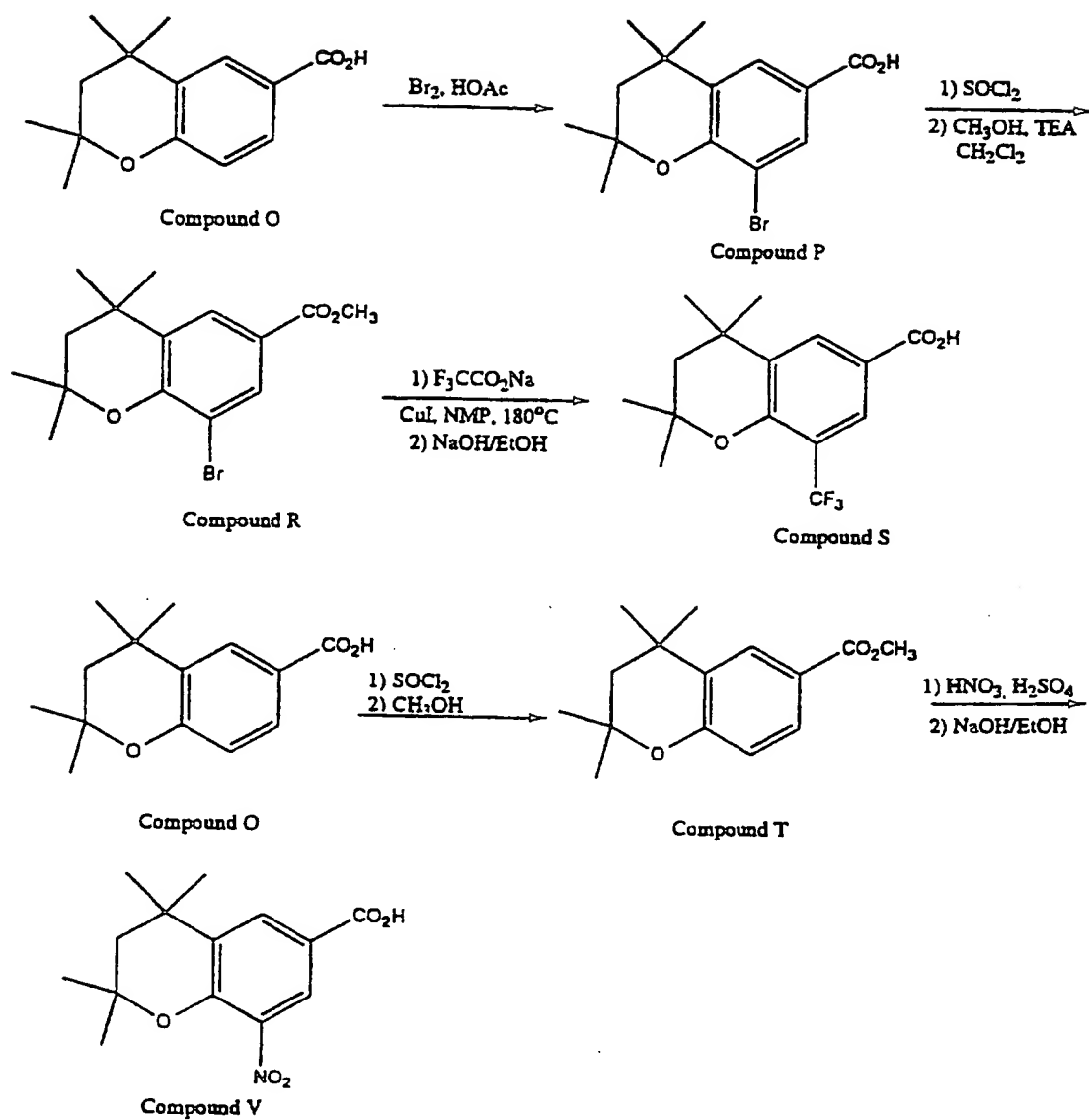chemical reactions or processes which _per se_ are
known in the art.

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Reaction Scheme 1

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Reaction Scheme 2

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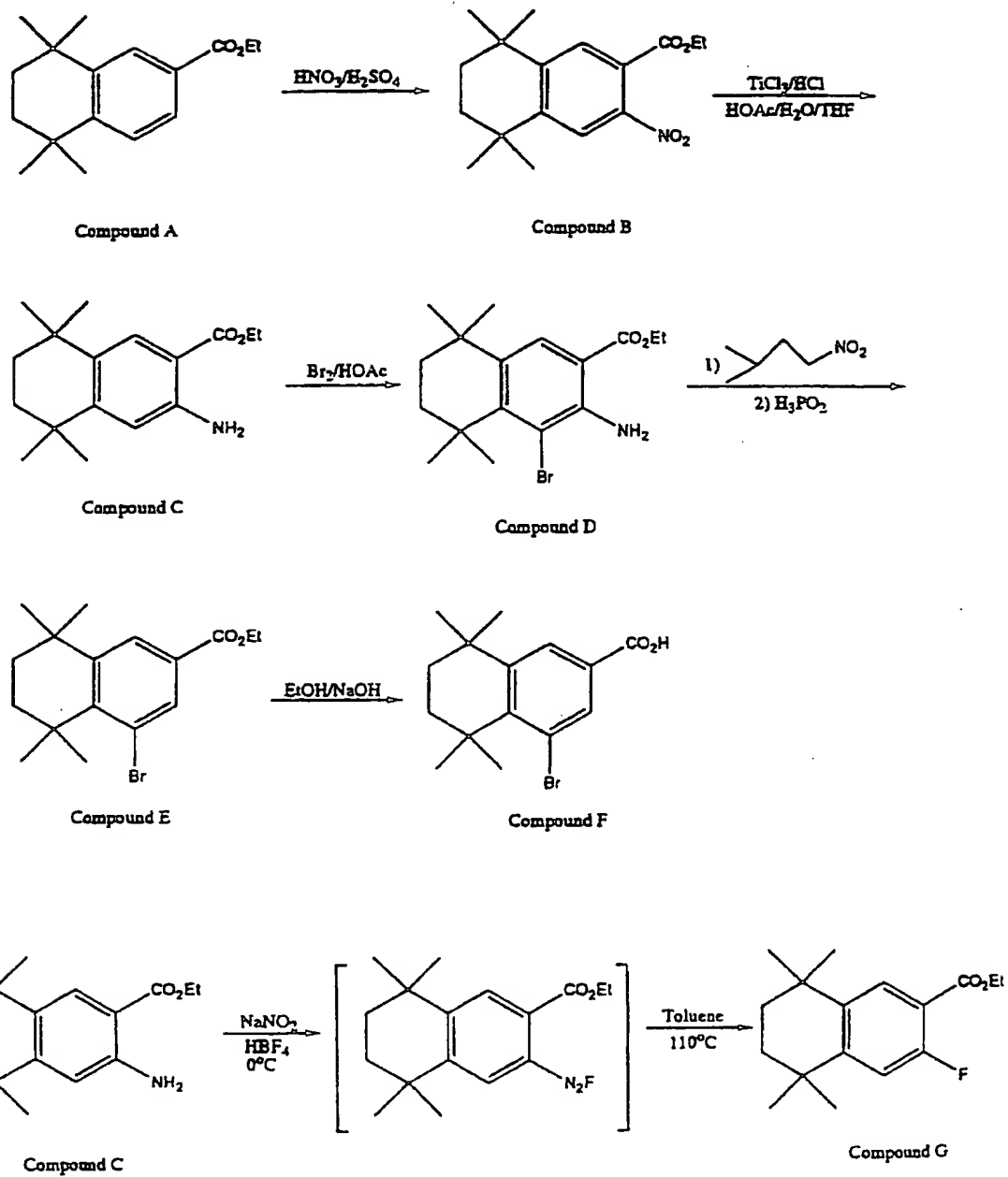
Reaction Scheme 2 (continued)

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1   Reaction Schemes 1 and 2 provide examples for

2 the synthesis of derivatives of 5,6,7,8-tetrahydro-

3 5,5,8,8-tetramethyl-naphthalene-2-carboxylic acid,

4 which are within the scope of Formula 6 and which

5 are reacted with an amine of Formula 7 to provide

6 (5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-naphthalene-

7 2-yl)carbamoyl derivatives within the scope of

8 Formula 1. Thus, as is shown in Reaction Scheme 1,

9 ethyl 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-

10 naphthalene-2-carboxylate (Compound A) is nitrated

11 to provide the corresponding 3-nitro compound

12 (Compound B). The nitro group of Compound B is

13 reduced to provide the corresponding 3-amino

14 compound (Compound C) which is described in the

15 publication Lehmann et al. Cancer Research, 1991,

16 51, 4804. Ethyl 5,6,7,8-tetrahydro-5,5,8,8-tetra-

17 methyl-3-amino-naphthalene-2-carboxylate (Compound

18 C) is brominated to yield the corresponding 4-bromo

19 derivative (Compound D), which is converted by

20 treatment with isoamylnitrite and reduction with

21 H_3PO_2, to ethyl 5,6,7,8-tetrahydro-5,5,8,8-tetra-

22 methyl- 4-bromonaphthalene-2-carboxylate (Compound

23 E). Saponification of Compound E yields

24 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-4-bromonaphth

25 alene-2-carboxylic acid (Compound F) which is used

26 as a reagent in accordance with Formula 6. Ethyl

27 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-aminonaphth

28 alene-2-carboxylate (Compound C) is also diazotized

29 and reacted with HBF_4 to provide ethyl

30 5,6,7,8-tetrahydro-5,5,8,8-tetra-methyl-3-fluoronaph

31 thalene-2-carboxylate (Compound G) which serves

32 either per se or after saponification as a reagent

33 in accordance with Formula 6.

34   5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-

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1 hydroxynaphthalene (**Compound H**, available in

2 accordance with the publication <u>Krause</u> **Synthesis**

3 **1972 140**), is the starting material in the example

4 shown in **Reaction Scheme 2**. **Compound H** is

5 brominated to provide the corresponding 3-bromo

6 compound (**Compound I**) which is thereafter protected

7 in the hydroxyl function by treatment with

8 methoxymethyl chloride (MOMCl) to yield

9 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-methoxymet-

10 hoxy-2-bromonaphthalene (**Compound J**). **Compound J** is

11 reacted with <u>t</u>-butyllithium and carbon dioxide to

12 provide the corresponding carboxylic acid (**Compound**

13 **K**) from which the methoxymethyl protecting group is

14 removed by acid to give

15 5,6,7,8-tetrahydro-5,5,8,8-tetra-

16 methyl-2-hydroxynaphthalene-3-carboxylic acid

17 (**Compound L**). **Compound L** is brominated to yield

18 5,6,7,8-tetrahy-

19 dro-5,5,8,8-tetramethyl-1-bromo-2-hydroxynaphthalene

20 -3-carboxylic acid (**Compound M**). **Compound L** and

21 **Compound M** serve as reagents in accordance with

22 **Formula 6**. The hydroxy group of **Compound M** is

23 protected for further transformations with

24 methoxymethyl chloride (MOMCl) in the presence of

25 base, yielding 5,6,7,8-tetrahydro-5,5,8,8-

26 tetramethyl-1-bromo-2-methoxymethoxynaphthalene-3-ca
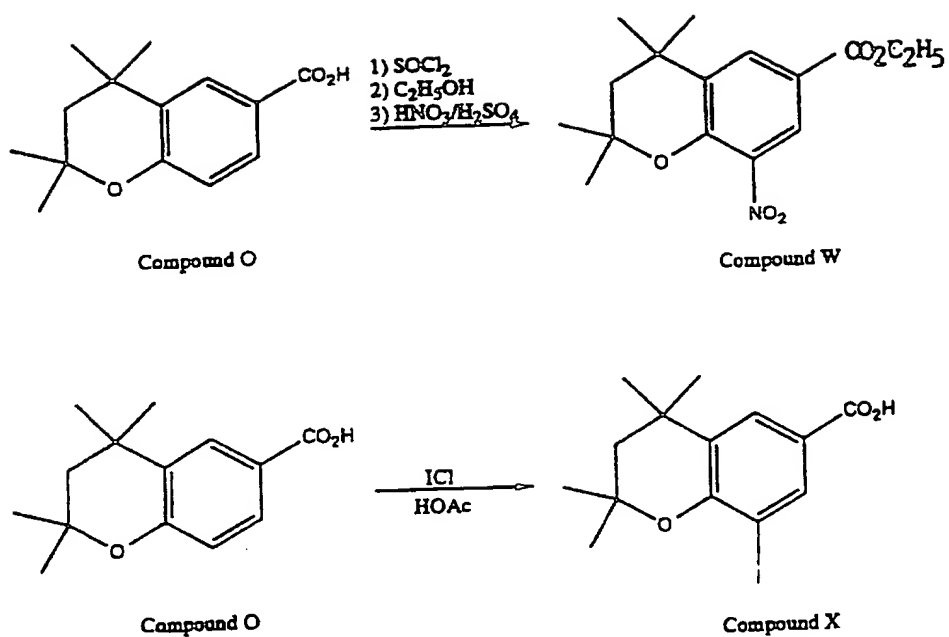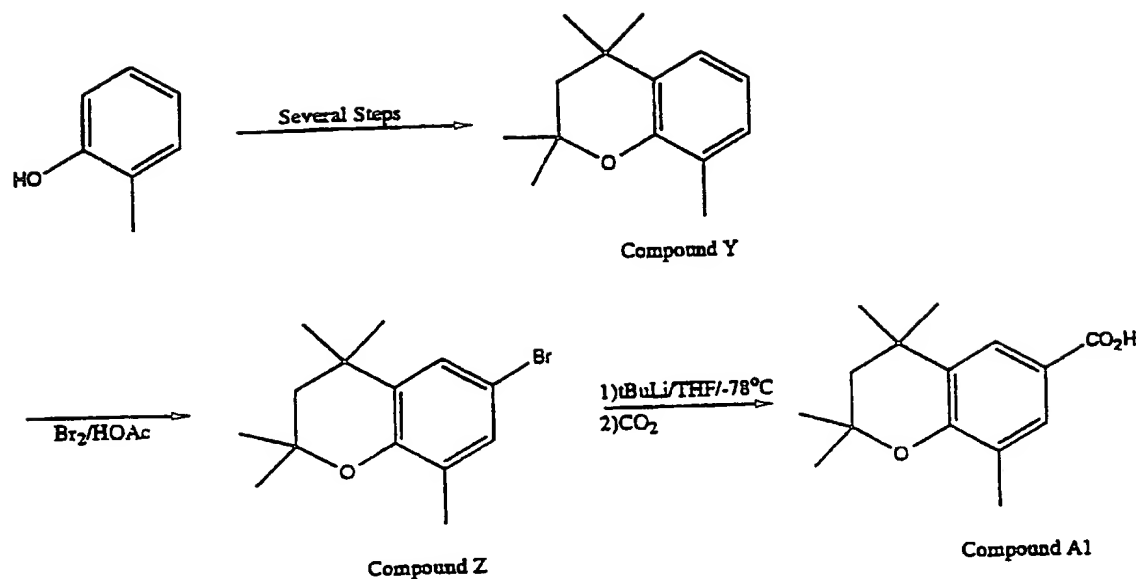
27 rboxylic acid (**Compound N**).

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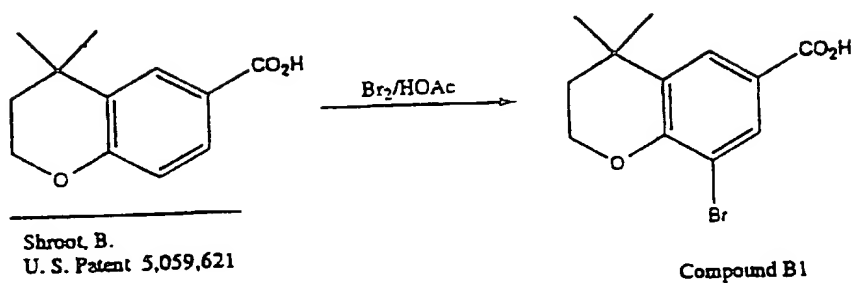21
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Compound O

1) SOCl₂
2) C₂H₅OH
3) HNO₃/H₂SO₄

Compound W

Compound O

ICl
HOAc

Compound X

Reaction Scheme 3

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Compound Y

Compound Z

Compound A1

Reaction Scheme 4

Shroot, B.
U. S. Patent 5,059,621

Compound B1

Reaction Scheme 5

51

1   Reaction Schemes 3, 4 and 5 provide examples for

2   the synthesis of derivativ s of 2,2,4,4 and

3   4,4-substituted chroman-6-carboxylic acids which can

4   serve as reagents in accordance with Formula 6 for

5   the synthesis of the carbamoyl (amide) compounds

6   within the scope of the present invention. Thus,

7   referring now to Reaction Scheme 3,

8   2,2,4,4-tetramethylchroman-6-carboxylic acid

9   (Compound O, see U. S. Patent No. 5,006,550) is

10 brominated with bromine in acetic acid to yield the

11 corresponding 8-bromo derivative (Compound P).

12 Compound P is converted to the acid chloride by

13 treatment with thionyl chloride, and the resulting

14 acid chloride is suitable for reaction with an amine

15 of Formula 3 to provide the carbamoyl (amide)

16 compounds of the invention. The acid chloride is

17 also reacted with an alcohol (methanol) in the

18 presence of base to yield the corresponding ester,

19 methyl 2,2,4,4-tetramethyl-8-bromochroman-6-

20 carboxylate (Compound R). The bromo function of

21 Compound R is converted to a trifluoromethyl

22 function by treatment with sodium trifluoroacetate

23 in the presence of cuprous iodide catalyst and

24 1-methyl-2-pyrrolidinone (NMP), and the carboxylate

25 ester group is saponified to yield

26 2,2,4,4-tetramethyl-8-trifluoromethylchroman-6-carbo

27 xylic acid (Compound S). Compound S is within the

28 scope of Formula 6 and is suitable per se or as the

29 acid chloride or in other "activated" form to react

30 with the amines of Formula 7 to yield the carbamoyl

31 (amide) compounds of the invention.

32 2,2,4,4-Tetramethylchroman-6-carboxylic acid

33 (Compound O) is also convert d to the methyl ester

34 (Compound T) which is then nitrated to yield

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1   2,2,4,4-tetramethyl-8-nitrochroman-6-carboxylic acid

2   (Compound V), still another reagent within the scope

3   of Formula 6. Moreover, in the example further

4   shown in Reaction Scheme 3,

5   2,2,4,4-tetramethylchroman- 6-carboxylic acid

6   (Compound O) is converted to the ethyl ester and

7   nitrated thereafter to yield ethyl

8   2,2,4,4-tetramethyl-8-nitrochroman-6-carboxylate

9   (Compound W). Still further, Compound O is reacted

10 with ICl to yield 2,2,4,4-tetramethyl8-iodochroman-

11 6-carboxylic acid (Compound X).

12   In accordance with the example shown in Reaction

13 Scheme 4, 2-methylphenol is subjected to a series of

14 reactions in accordance with the teachings of United

15 States Patent No. 5,045,551 (incorporated herein by

16 reference) to yield 2,2,4,4,8-pentamethylchroman

17 (Compound Y). Compound Y is brominated with bromine

18 in acetic acid to give 2,2,4,4,8-pentamethyl-6-

19 bromochroman (Compound Z) which is reacted with

20 $\underline{t}$-butyl lithium and thereafter with carbon dioxide

21 to give 2,2,4,4,8-pentamethylchroman-6-carboxylic

22 acid (Compound A_1).

23   Reaction Scheme 5 illustrates the synthesis of

24 4,4-dimethyl-8-bromochroman-6-carboxylic acid

25 (Compound B_1) by bromination of

26 4,4,-dimethyl-chroman-6-carboxylic acid which is

27 available in accordance with the teachings of United

28 States Patent No. 5,059,621, the specification of

29 which is incorporated herein by reference.

30 2,2,4,4,8-Pentamethylchroman-6-carboxylic acid

31 (Compound A_1) and 4,4,-dimethyl-8-bromochroman-

32 6-carboxylic acid (Compound B_1) serve as reagents,

33 either $\underline{per\ se}$, or as the corresponding acid

34 chlorides (or other "activated form), in accordance

with Formula 6 for the synthesis of the carbamoyl
(amide) compounds of the present invention.

Referring back now to the reaction between the
reagent of Formula 6 with an amine compound of
Formula 7 it is noted that the amine compounds are,
generally speaking, available in accordance with the
state-of-the-art. as described in the scientific and
patent literature. More specifically, the amine
compounds of Formula 7 can be prepared as described
in the scientific and patent literature, or from
known compounds of the literature, by such chemical
reactions or transformations which are within the
skill of the practicing organic chemist. Reaction
Scheme 6 illustrates examples for the preparation of
amine compounds of Formula 7 (where Y is phenyl)
from commercially available starting materials
(Aldrich Chemical Company, or Research Plus, Inc.).
The illustrated compounds of Formula 7 are used for
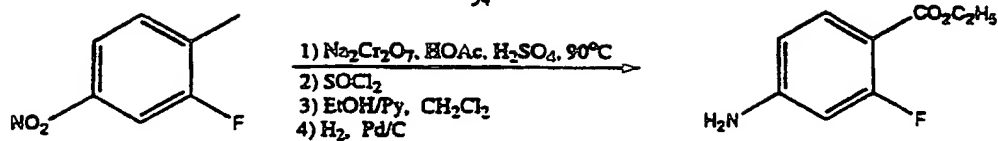the synthesis of several preferred compounds used in
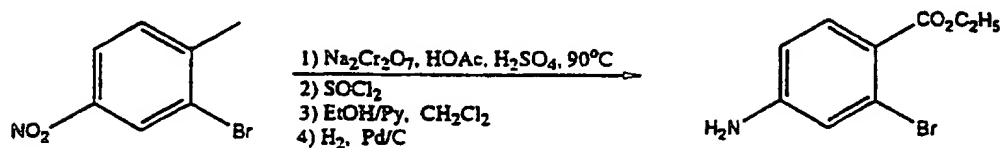the methods of the invention.

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1) Na$_2$Cr$_2$O$_7$, HOAc, H$_2$SO$_4$, 90°C
2) SOCl$_2$
3) EtOH/Py, CH$_2$Cl$_2$
4) H$_2$, Pd/C

Compound C1

1) Na$_2$Cr$_2$O$_7$, HOAc, H$_2$SO$_4$, 90°C
2) SOCl$_2$
3) EtOH/Py, CH$_2$Cl$_2$
4) H$_2$, Pd/C

Compound D1

1) Na$_2$Cr$_2$O$_7$, HOAc, H$_2$SO$_4$, 90°C
2) SOCl$_2$
3) EtOH/Py, CH$_2$Cl$_2$
4) H$_2$, Pd/C

Compound E1

1) SOCl$_2$
2) MeOH/TEA/ CH$_2$Cl$_2$

Compound F1

EDC, DMAP
EtOH

Compound G1

1) SOCl$_2$
2) CH$_3$OH/Py
3) NaN$_3$/CH$_3$CN
4) H$_2$, Pd/C

Reaction Scheme 6

Compound H1

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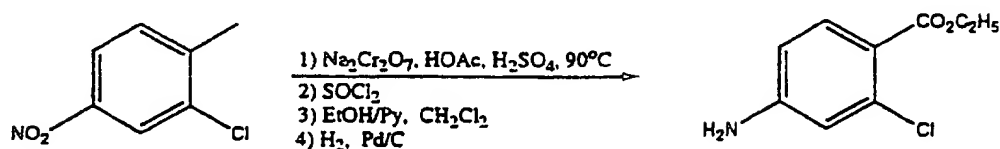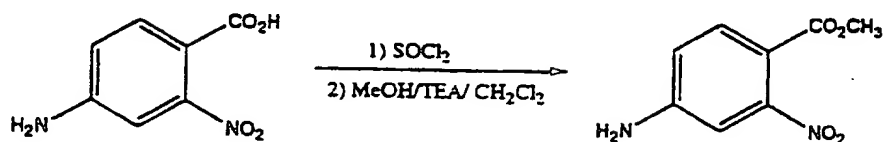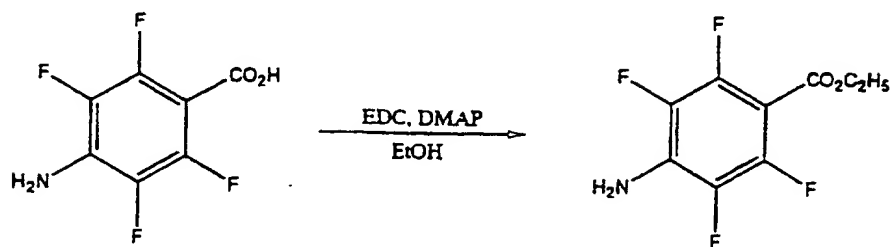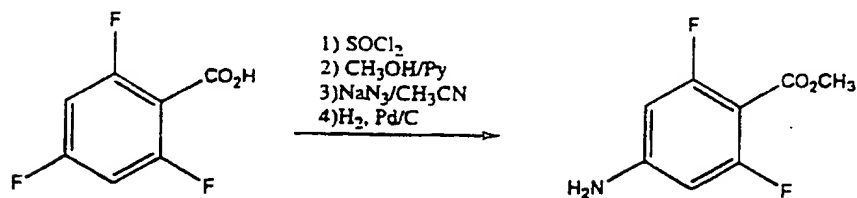
1   Thus, in accordance with Reaction Scheme 6,
2   3-nitro-6-methyl-fluorobenzene (Aldrich) is
3   subjected to oxidation, conversion of the resulting
4   carboxylic acid to an acid chloride and thereafter
5   to an ethyl ester, followed by reduction of the
6   nitro group, to yield ethyl
7   2-fluoro-4-amino-benzoate (Compound C_1).
8   3-Nitro-6-methyl-bromobenzene (Aldrich) and
9   3-nitro-6-methyl-chlorobenzene (Aldrich) are
10 subjected to essentially to the same series of
11 reactions to yield ethyl 2-bromo-4-amino-benzoate
12 (Compound D_1) and ethyl 2-chloro-4-amino-benzoate
13 (Compound E_1), respectively. 2-Nitro-4-aminobenzoic
14 acid (Research Plus) is converted to its methyl
15 ester (Compound F_1) through the corresponding acid
16 chloride. 2,3,5,6-Tetrafluoro-4-amino-benzoic acid
17 (Aldrich) is esterified by treatment with ethanol in
18 the presence of 1-(3-dimethylaminopropyl)-3-
19 ethylcarbodiimide hydrochloride (EDC) and
20 4-dimethylaminopyridine in CH_2Cl_2 to give ethyl
21 2,3,5,6-tetrafluoro-4-amino-benzoate (Compound G_1).
22 2,4,6-Trifluorobenzoic acid (Aldrich) is converted
23 to the methyl ester through the acid chloride, and
24 the 4-fluoro atom is displaced by reaction with
25 sodium azide, followed by hydrogenation, to yield
26 methyl 2,6-difluoro-4-amino benzoate (Compound H_1).
27 Compounds C_1, D_1, E_1, F_1, G_1 and H_1 serve as amine
28 reagents in accordance with Formula 7. Further
29 examples of reagents in accordance with Formula 7
30 are nitro, fluoro, chloro, bromo and trifluoromethyl
31 derivatives of amino substituted heteroaryl
32 carboxylic acids, or their lower alkyl esters, such
33 as ethyl 2-amino-4-chloropyridine 2-carboxylate,
34 ethyl 5-amino-3-chloropyridine 5-carboxylate, and

1 3,4-dibromo-5-aminothiophene-2-carboxylic acid.   Th

2 latter examples qcan be prepared by respective

3 chlorination or bromination of

4 2-aminopyridine-5-carboxylic acid or of its ester,

5 3-aminopyridine-6-carboxylic acid or of its ester

6 (described in WO 93/06086) and of

7 2-aminothiophene-5-carboxylic acid (described in

8 PCT/US92/06485).

9   The reactions between the compounds of **Formula 6**

10 and **Formula 7** or between compounds of **Formula 6a** and

11 **7a**, described above, comprise the actual syntheses

12 of the carbamoyl (amide) compounds of the invention.

13 Numerous examples of this reaction are described in

14 detail in the experimental section below. The

15 carbamoyl (amide) compounds of the invention can be

16 converted into thiocarbamoyl (thioamide) compounds

17 of the invention where with reference to **Formula 1 Z**

18 is S, by reacting the carbamoyl (amide) compound

19 with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-

20 diphosphetane-2,4-disulfide (Lawesson's reagent).

21 This reaction is illustrated in **Reaction Scheme 7**

22 for two specific examples for the compounds used in

23 the methods of the invention.
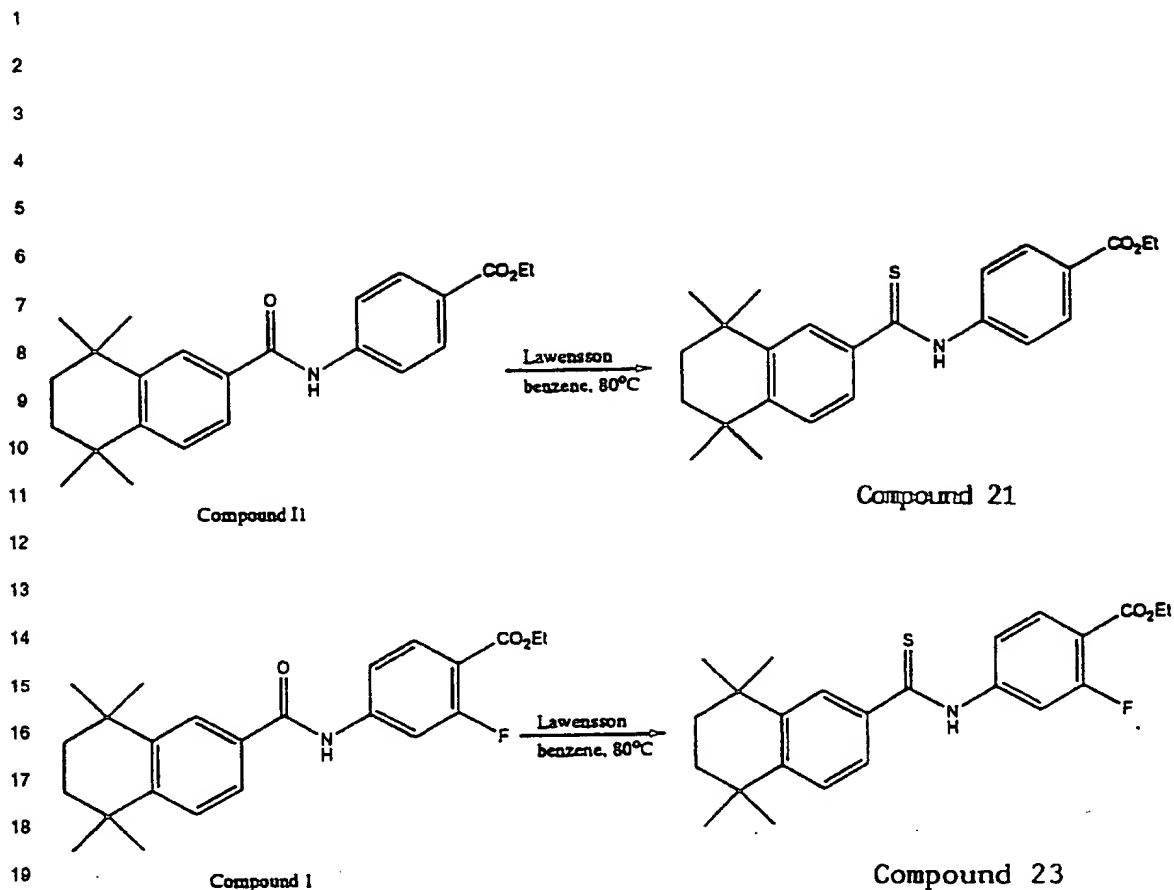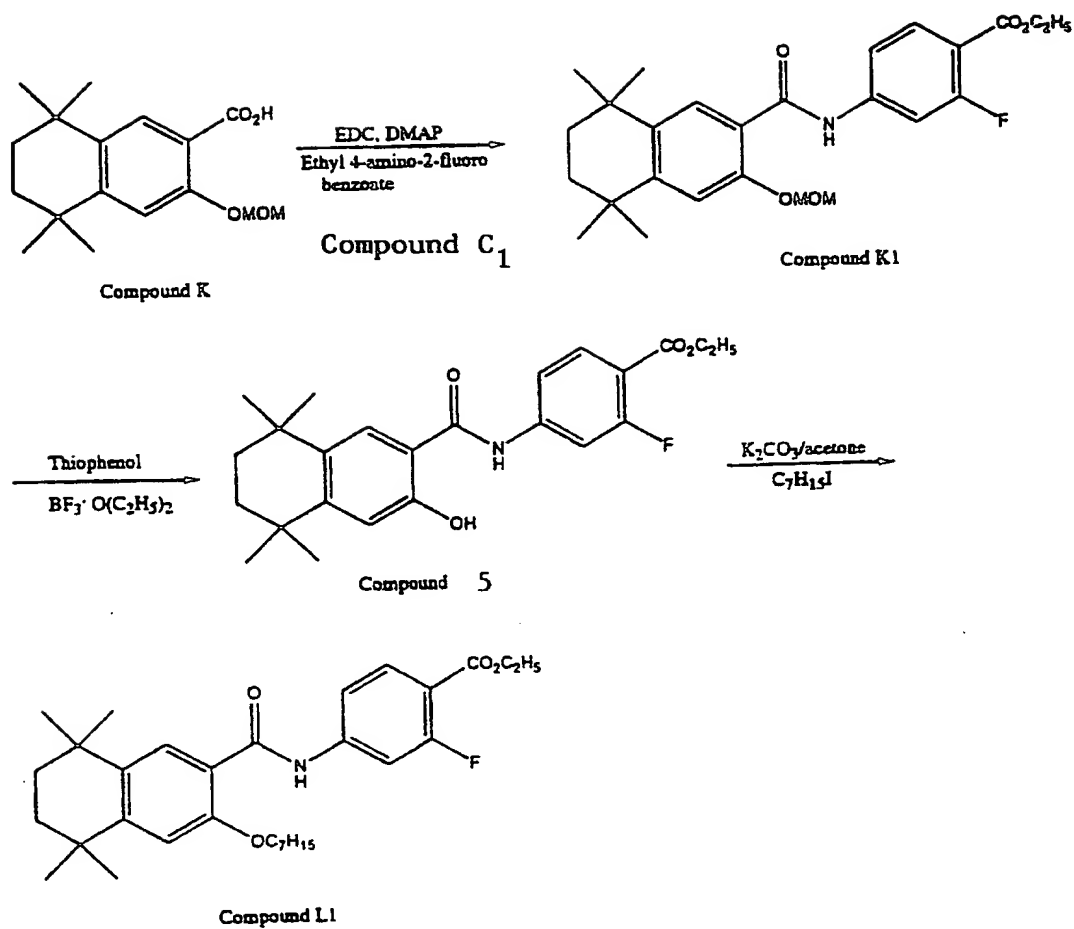
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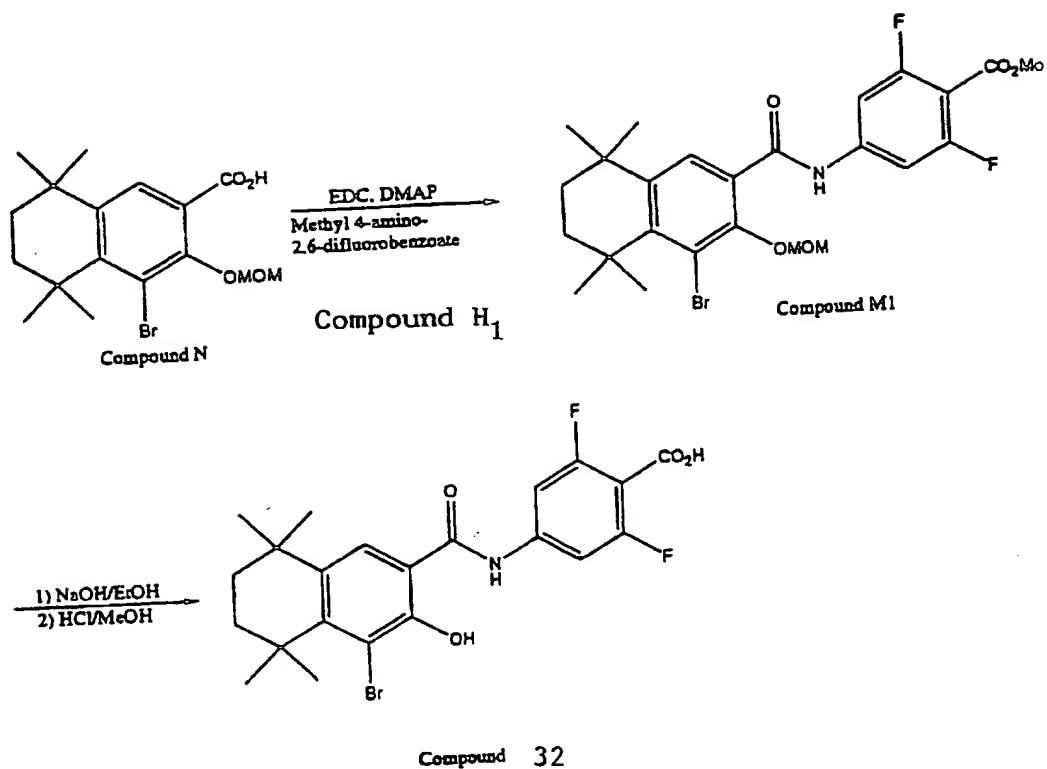25

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Compound I1

Lawensson
benzene, 80°C

Compound 21

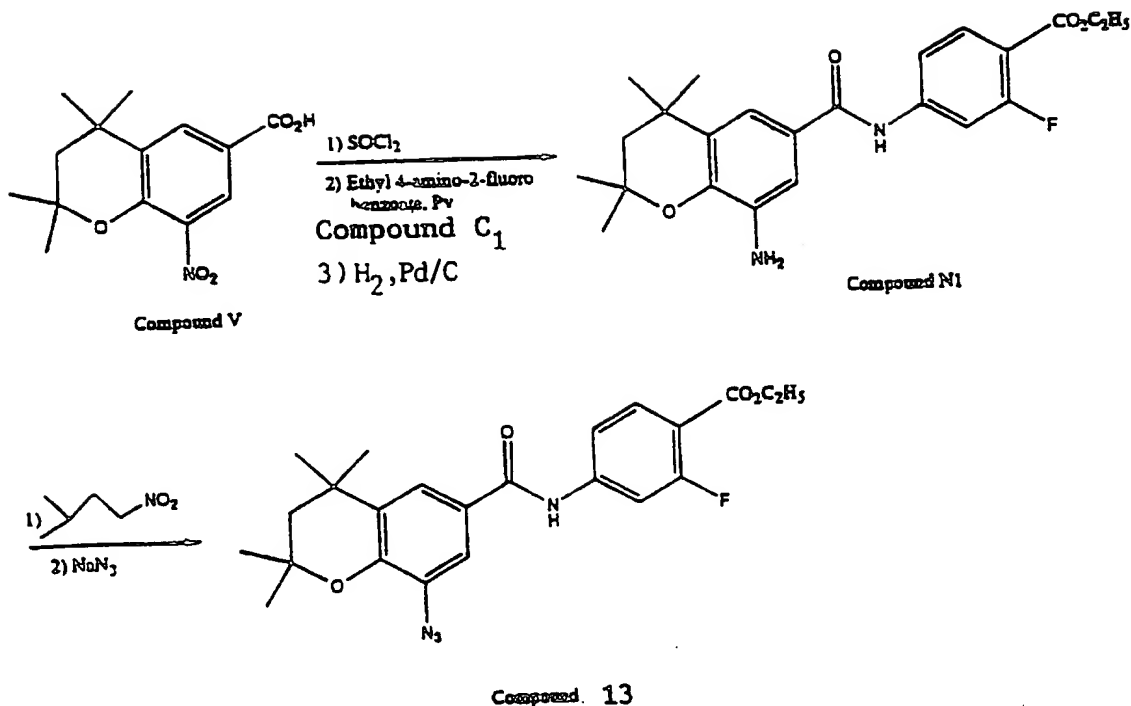Compound I

Lawensson
benzene, 80°C

Compound 23

Reaction Scheme 7

In Reaction Scheme 7 one starting material ethyl
4-[5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-
naphthalen-2-yl)carbamoyl]benzoate (Compound I₁) is
obtained in accordance with the teachings of
Kagechika et al. J. Med Chem. 1988 31, 2182 - 2192.
The other starting material, ethyl
2-fluoro-4-[5',6',7',8'-tetrahydro-5',5',8',8'-tetra
methylnaphthalen-2-yl)carbamoyl]benzoate (Compound
1) is obtained in accordance with the present
invention.

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Compound C_1

Compound K

Compound K1

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Compound 5

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Compound L1

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Reaction Scheme 8

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13   EDC. DMAP

14   Methyl 4-amino-
            2,6-difluorobenzoate

Compound H₁

Compound N   Compound M1

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21   1) NaOH/EtOH

22   2) HCl/MeOH

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24   Compound 32

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34   **Reaction Scheme 9**
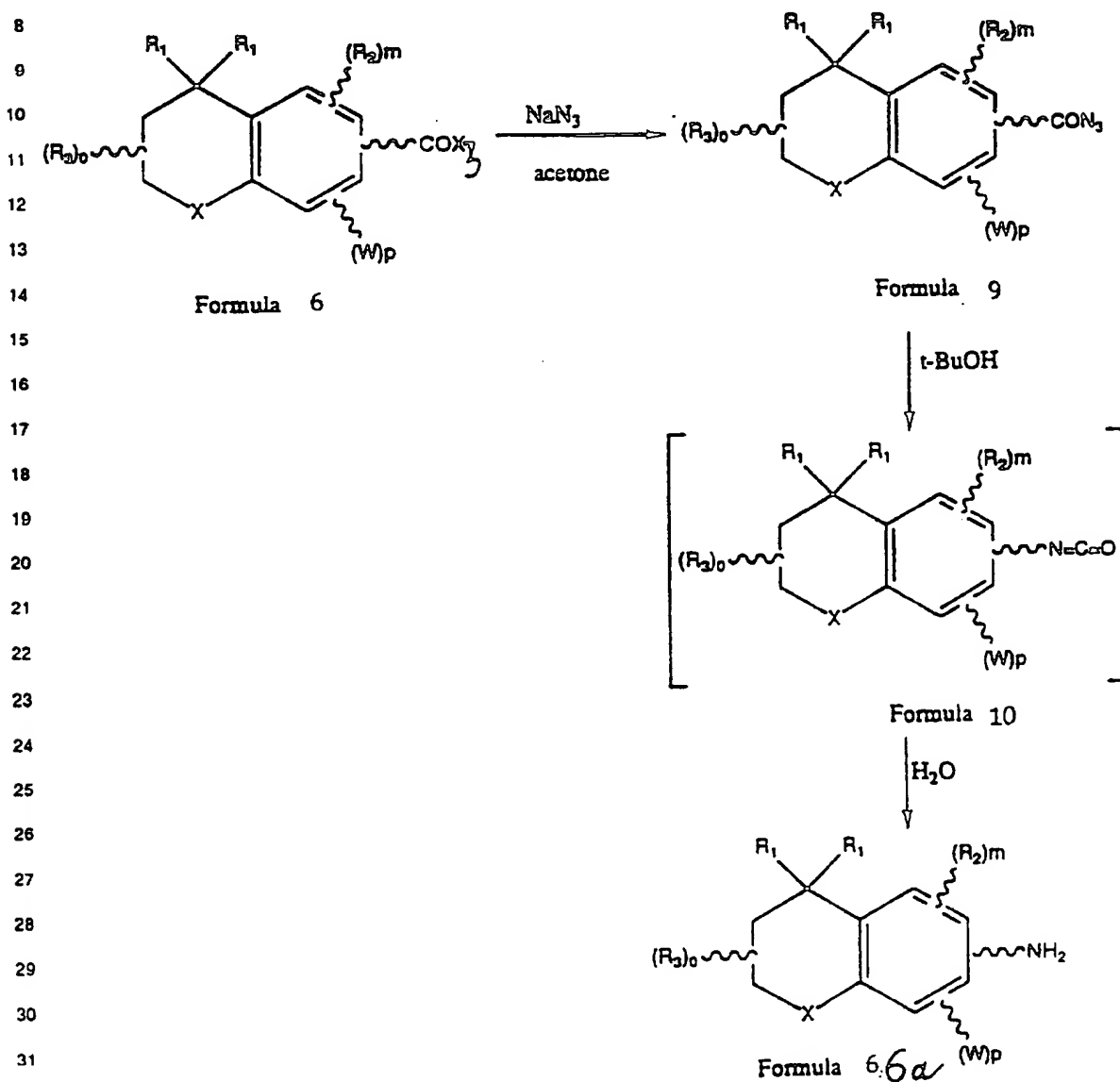
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Reaction Scheme 10

Reaction Schemes 8, 9 and 10 disclose examples
for the preparation of carbamoyl (amide) compounds
of the invention, first by a coupling reaction of a
compound of Formula 6 with a compound of Formula 7,
followed by one or more reactions performed on the
carbamoyl (amide) compound that has been first
obtained directly in the coupling reaction. Thus,
as is shown in Reaction Scheme 8,
5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-
3-methoxymethoxynaphthalene-2-carboxylic acid
(Compound K) is coupled with ethyl
4-amino-2-fluorobenzoate (Compound C_1) in CH_2Cl_2 in
the presence of 1-(3-dimethylaminopropyl)-3-
ethylcarbodiimide hydrochloride (EDC) and
dimethylaminopyridine (DMAP) to give ethyl

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1 2-fluoro-4-[5',6',7',8'-tetrahydro-5',5',8',8'-tetra

2 methyl-2'-m thoxymethoxy-naphthalen-

3 3'-yl)carbamoyl]benzoate (Compound K₁). The

4 methoxymethyl protecting group is removed from

5 Compound K₁ by treatment with thiophenol and

6 borontrifluoride ethereate resulting in ethyl

7 2-fluoro-4-[5',6',7',8'-tetrahydro-5',5',8',8'-tetra

8 methyl-2'-hydroxy-naphthalen-3'-yl)carbamoyl]-

9 benzoate (Compound 5). The hydroxy function of

10 Compound 5 is converted into an n-hexyl ether by

11 treatment with hexyl iodide in the presence of mild

12 base.

13   In accordance with Reaction Scheme 9

14 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-1-bromo-2-met

15 hoxymethoxynaphthalene-3-carboxylic acid (Compound

16 N) is coupled with methyl 4-amino-2,6-difluoro-

17 benzoate (Compound B₁) in CH₂Cl₂ solvent in the

18 presence of ethylcarbodiimide hydrochloride (EDC)

19 and DMAP to provide methyl

20 2,6-difluoro-4-[(5',6',7',8'-tetrahydro-5',5',8',8'-

21 tetramethyl-1'-bromo-2'-methoxymethoxy-naphthalen-3'

22 -yl)carbamoyl]benzoate (Compound M₁), from which the

23 esterifying methyl group and the methoxymethyl

24 protecting group are removed by treatment with base

25 and acid, respectively to yield

26 2,6-difluoro-4-[(5',6',7',8'-tetrahydro-5',5',8',8'-

27 tetramethyl-1'-bromo-2'-hydroxy-naphthalen-3'-yl)car

28 bamoyl]benzoic acid (Compound 32).

29   Reaction Scheme 10 discloses the example of

30 converting 2,2,4,4-tetramethyl-8-nitrochroman-6-

31 carboxylic acid (Compound V) into the corresponding

32 acid chloride by treatment with thionyl chloride,

33 followed by coupling with ethyl

34 4-amino-2-fluorobenzoate (Compound C₁) and

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1 hydrogenation to yi ld ethyl

2 2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-amino-6'-chr

3 omanyl)carbamoyl]benzoate (Compound N₁). Compound N₁

4 is converted to the corresponding 8-azido compound,

5 ethyl 2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-azido-

6 6'-chromanyl)carbamoyl]benzoate (Compound 13) by

7 treatment with isoamyl nitrate and NaN₃.

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14   Formula 6   Formula 9

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16   t-BuOH
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23   Formula 10
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25   H₂O
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31   Formula 6.6a   (W)p
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34   Reaction Scheme 11

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1   Reaction Scheme 11 illustrates the synthesis of
2   the primary amine compounds of Formula 6a from the
3   acid chlorides (X_3 = Cl) or other form of activated
4   acids of Formula 6 where the primary amine of
5   Formula 6a is not available by a published
6   literature procedure. Thus, substantially in
7   accordance with the step of a Curtius rearrangement,
8   the acid chloride of Formula 6 is reacted with
9   sodium azide in acetone to yield the azide compound
10   of Formula 9. The azide of Formula 9 is heated in a
11   polar high boiling solvent, such as t-butanol, to
12   provide the intermediate isocyanate of Formula 10,
13   which is hydrolyzed to yield a compound of Formula
14   6a.

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21   Sugawara, S; Ishikawa, N.
22   *Kogyo Kagaku Zasshi*
         1970, 73, 972-979
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30   Michael Reuman *et al*
         J. Med. Chem.
31   1995, 38, 2531-2540
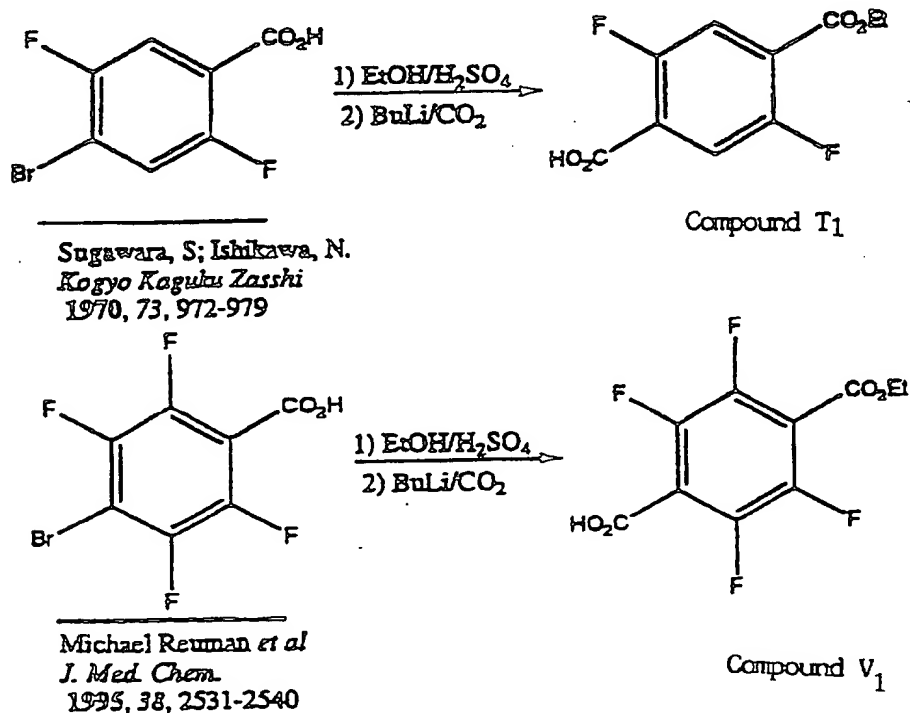
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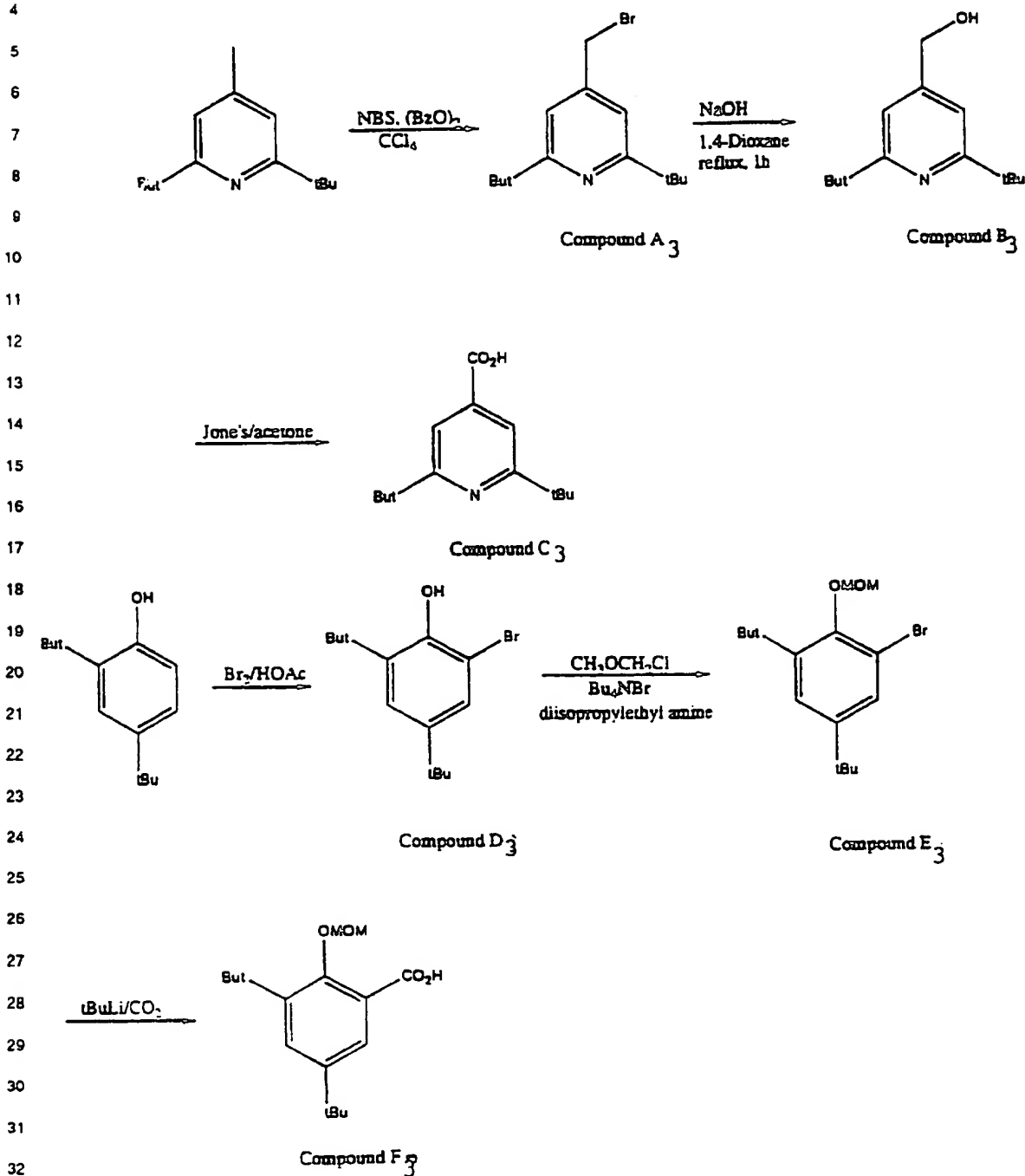34   Reaction Scheme 12

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Reaction Scheme 12 illustrates examples for
preparing compounds of Formula 7a where such
compounds are not available commercially or by a
published literature procedure. Thus, by way of
example 2,5-difluoro-4-bromobenzoic acid (available
by the literature procedure of Sugawara et al. Kogyo
Kaguku Zasshi 1970, 73, 972-979) is first esterified
by treatment with ethyl alcohol and acid to yield
the corresponding ester, and thereafter is reacted
with butyl lithium followed by carbon dioxide to
give the monoester of 2,5-difluoro terephthalic acid
(Compound T_1). A similar sequence of reactions
performed on 2,3,5,6-difluoro-4-bromobenzoic acid
(available by the literature procedure of Reuman et
al. J. Med. Chem. 1995, 38, 2531-2540) yields the
monoester of 2,3,5,6-tetrafluoroterephthalic acid
(Compound V_1). The just illustrated sequence of
reaction can be, generally speaking, utilized for
the synthesis of all compounds of Formula 7a with
such modification which will become readily apparent
to those skilled in the art, where such compounds
are not available by a known literature procedure.

Reaction Scheme 13 provides an example for the
preparation of 2,6-di-tert-butylisonicotinic acid
(Compound C_3) which is a reagent in accordance with
Formula 8 for the preparation of several preferred
compounds of the present invention. Thus,
2,6-di-tert-butyl-4-methylpyridine (available
commercially from Aldrich Chemical Co.) is reacted
with N-bromosuccinimide and benzoyl peroxide to
provide 4-bromomethyl-2,6-di-tert-butylpyridine
(Compound A_3). Compound A_3 is reacted with base
(sodium hydroxyde) to yield the coresponding
hydroxymethyl compound (Compound B_3), which is

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thereafter oxidized in a Jones oxidation reaction to
give 2,6-di-tert-butylisonicotinic acid (**Compound
C₃**).

Compound A₃

Compound B₃

Compound C₃

Compound D₃

Compound E₃

Compound F₃

Reaction Scheme 13

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1   A further example of a compound which serves as

2   a reagent for preparing the carbamoyl (or amide)

3   compounds of the present invention is provided in

4   Reaction Scheme 13.   2,4-Di-tert-butylphenol

5   (Aldrich) is brominated in glacial acetic acid to

6   yield 2-bromo-4,6-di-tert-butylphenol (Compound D_3)

7   which is thereafter reacted with methoxymethyl

8   chloride (MOMCl)to give

9   O-methoxymethyl-2-bromo-4,6-di-tert-butylphenol

10 (Compound E_3). Compound E_3 is treated with t-butyl

11 lithium followed by carbon dioxide to yield

12 O-methoxymethyl-3,5-di-tert-butylsalicylic acid

13 (Compound F_3). Compound F_3 is a reagent which

14 differs from the compounds generally encompassed by

15 Formula 8 only in that the hydroxyl funtion of this

16 compound is protected by the methoxymethyl (MOM)

17 group. However, the methoxymethyl protecting group

18 is removed after formation of the carbamoyl (amide)

19 linkage, as exemplified in Reaction Scheme 14.

20 Reaction of an aromatic bromo compound (such as

21 Compound D_3) with t-butyl lithium followed by carbon

22 dioxide is a preferred method for preparing several

23 aromatic carboxylic acids in accordance with Formula

24 8 and Formula 7a, described in the present

25 application.

26   The primary amine compounds of Formula 8a which

27 are not available commercially or by a published

28 literature procedure can be made from the acid

29 chlorides (X_3 = Cl) or other form of activated acids

30 of Formula 8 substantially in accordance with the

31 steps of a Curtius rearrangement, in analogy to the

32 reaction steps d scribed above in connection with
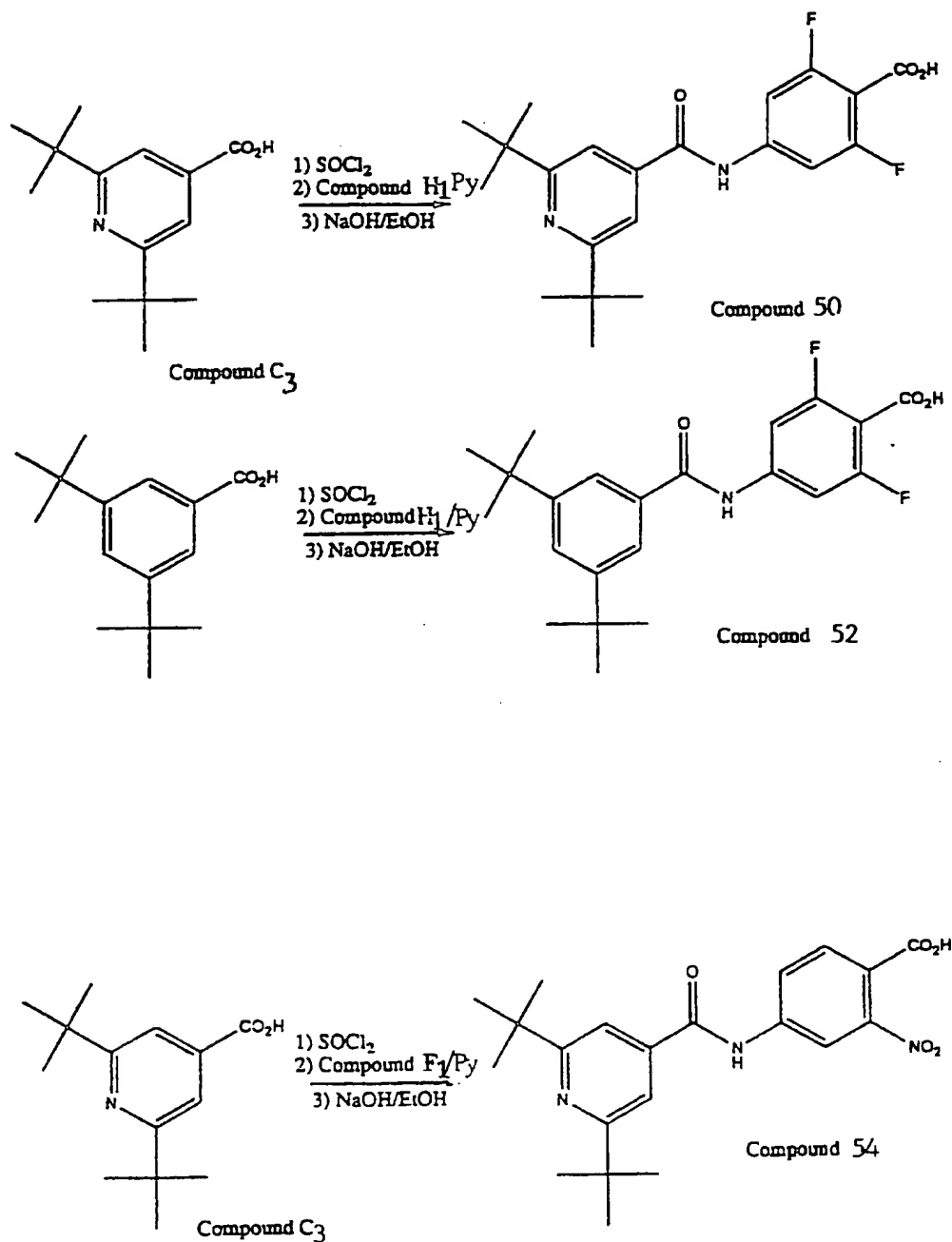
33 Reaction Scheme 11.
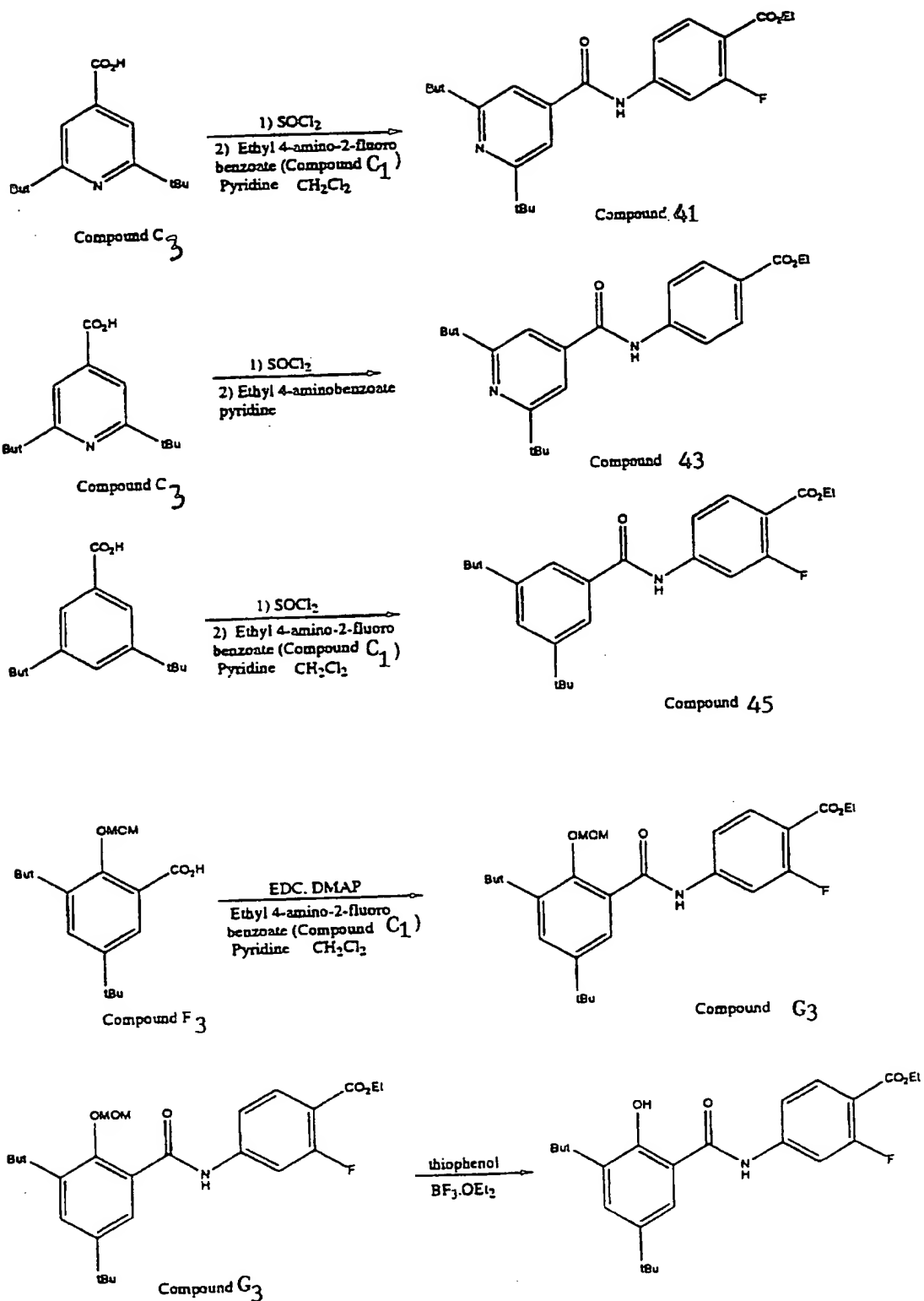
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Reaction Scheme 14

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Reaction Scheme 14 (continued)

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Reaction Scheme 14 illustrat s examples for the
formation of the carbamoyl (amide) compounds in
accordance with Formula 2, by reaction of a reagent
of Formula 8 with a reagent of Formula 7. Thus,
2,6-di-tert-butylisonicotinic acid (Compound C_3) is
reacted with thionyl chloride ($SOCl_2$) to provide the
intermediate acid chloride, which is then reacted
with ethyl 2-fluoro-4-amino-benzoate (Compound C_1) in
the presence of an acid acceptor (pyridine) to yield
ethyl 2-fluoro-4-[(2'6'-di-tert-butylpyrid-4'-
yl)carbamoyl]benzoate (Compound 41). As another
example, 3,5-di-tert-butylbenzoic acid (available by
the literature procedure of Kagechika et al., J.
Med. Chem. 1988, 31, 2182, incorporated herein by
reference) is reacted with thionyl chloride,
followed by ethyl 2-fluoro-4-amino-benzoate
(Compound C_1) to yield ethyl 2-fluoro-4-[(3',5'-di-
tert-butylphenyl)carbamoyl]benzoate (Compound 45).
As still another example, O-methoxymethyl-3,5-di-
tert-butylsalicylic acid (Compound F_3)is reacted with
ethyl 2-fluoro-4-amino-benzoate (Compound C_1) in the
presence of 4-dimethylaminopyridine (DMAP) catalyst
and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
hydrochloride (EDC) to give ethyl 2-fluoro-4-[(2'-
methoxymethyl-3',5'-di-tert-butylphenyl)car-
bamoyl]benzoate (Compound G_3). The methoxymethyl
protecting group is removed from Compound G_3 by
treatment with borontrifluoride ethereate and
thiophenol to yield ethyl 2-fluoro-4-[(2'-hydroxy-
3',5'-di-tert-butylphenyl)carbamoyl]benzoate
(Compound 47).

In yet another example shown in Reaction Scheme
14, 2,6-di-tert-butylisonicotinic acid (Compound C_3)
is reacted with thionyl chloride ($SOCl_2$), the

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1   resulting intermediate acid chloride is reacted with

2   methyl 2,6-difluoro-4-amino benzoate (Compound H₁),

3   followed by saponification of the ester group, to

4   yield 2,6-difluoro-4-[(2',6'-di-tert-butylpyrid-

5   4'yl)carbamoyl]benzoic acid (Compound 50).

6   3,5-Di-tert-butylbenzoic acid is subjected to the

7   same sequence of reactions to provide

8   2,6-difluoro-4- [(3',5'-di-tert-butylphenyl)car-

9   bamoyl]benzoic acid (Compound 52).

10   As yet another example, shown in Reaction Scheme

11   14, 2,6-di-tert-butylisonicotinic acid (Compound C₃)

12   is reacted with thionyl chloride (SOCl₂), followed by

13   methyl 2-nitro-4-aminobenzoate (Compound F₁) and

14   saponification of the ester function to give

15   2-nitro-4-[(2',6'-di-tert-butylpyrid-4'-yl)carbamoyl

16   ]benzoic acid (Compound 54).

17   Numerous other reactions suitable for preparing

18   compounds of the invention, and for converting

19   compounds of Formula 1 and/or of Formula 2 into

20   still further compounds which can be used in the

21   methods of treatment of the present invention, and

22   also for preparing the reagents of Formula 6,

23   Formula 7, Formula 8, Formula 6a, Formula 7a and

24   Formula 8a will become readily apparent to those

25   skilled in the art in light of the present

26   disclosure. In this regard the following general

27   synthetic methodology, applicable for conversion of

28   the compounds of Formula 1 and/or of Formula 2 into

29   further homologs and/or derivatives, and also for

30   preparing the reagents of Formula 6, Formula 7, and

31   8, (as well as 6a, 7a and 8a) is noted.

32   Carboxylic acids are typically esterified by

33   refluxing the acid in a solution of the appropriate

34   alcohol in the presence of an acid catalyst such as

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1   hydrogen chloride or thionyl chloride.
2   Alternatively, the carboxylic acid can be condensed
3   with the appropriate alcohol in the presence of
4   dicyclohexylcarbodiimide and dimethylaminopyridine.
5   The ester is recovered and purified by conventional
6   means. Acetals and ketals are readily made by the
7   method described in March, "Advanced Organic
8   Chemistry," 2nd Edition, McGraw-Hill Book Company, p
9   810). Alcohols, aldehydes and ketones all may be
10 protected by forming respectively, ethers and
11 esters, acetals or ketals by known methods such as
12 those described in McOmie, Plenum Publishing Press,
13 1973 and Protecting Groups, Ed. Greene, John Wiley &
14 Sons, 1981.
15   The acids and salts derived from compounds of
16 Formula 1 and Formula 2 are readily obtainable from
17 the corresponding esters. Basic saponification with
18 an alkali metal base will provide the acid. For
19 example, an ester may be dissolved in a polar
20 solvent such as an alkanol, preferably under an
21 inert atmosphere at room temperature, with about a
22 three molar excess of base, for example, potassium
23 or lithium hydroxide. The solution is stirred for
24 an extended period of time, between 15 and 20 hours,
25 cooled, acidified and the hydrolysate recovered by
26 conventional means.
27   The amide (in Formula 1 or 2 B is $CONR_9R_{10}$) may
28 be formed by any appropriate amidation means known
29 in the art from the corresponding esters or
30 carboxylic acids. One way to prepare such compounds
31 is to convert an acid to an acid chloride and then
32 tr at that compound with ammonium hydroxide or an
33 appropriate amine.
34   Alcohols are made by converting the

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corresponding acids to the acid chloride with
thionyl chloride or other means (J. March, "Advanced
Organic Chemistry", 2nd Edition, McGraw-Hill Book
Company), then reducing the acid chloride with
sodium borohydride (March, Ibid, pg. 1124), which
gives the corresponding alcohols. Alternatively,
esters may be reduced with lithium aluminum hydride
at reduced temperatures. Alkylating these alcohols
with appropriate alky halides under Williamson
reaction conditions (March, Ibid, pg. 357) gives the
corresponding ethers. These alcohols can be
converted to esters by reacting them with
appropriate acids in the presence of acid catalysts
or dicyclohexylcarbodiimide and
dimethylaminopyridine.

Aldehydes can be prepared from the corresponding
primary alcohols using mild oxidizing agents such as
pyridinium dichromate in methylene chloride (Corey,
E. J., Schmidt, G., Tet. Lett., 399, 1979), or
dimethyl sulfoxide/oxalyl chloride in methylene
chloride (Omura, K., Swern, D., Tetrahedron, 1978,
34, 1651).

Ketones can be prepared from an appropriate
aldehyde by treating the aldehyde with an alkyl
Grignard reagent or similar reagent followed by
oxidation.

Acetals or ketals can be prepared from the
corresponding aldehyde or ketone by the method
described in March, Ibid, p 810.

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Specific Examples

Ethyl 4-Amino-2-fluorobenzoate (Compound C_1)

To a mixture of 2-fluoro-4-nitrotoluene (1.0 g, 6.4 mmol, Aldrich) and $Na_2Cr_2O_7$ (2.74 g, 8.4 mmol) in 13.7 ml of HOAc was added slowly 6.83 ml of H_2SO_4. This mixture was slowly heated to 90 °C for 1 h to give a greenish heterogeneous solution. The mixture was cooled to room temperature and diluted with ethyl acetate. The PH of the solution was adjusted to 4 with NaOH (aq.). The mixture was extracted with more ethyl acetate. The organic layer was washed with $NaHCO_3$ (sat.), then brine and dried over Na_2SO_4. After filtration, the solution was concentrated to dryness which then was dissolved in 6 ml of $SOCl_2$, and heated at 80 °C for 1 h. The excess of $SOCl_2$ was removed under reduced pressure and the residue was dissolved in 5 ml of CH_2Cl_2, 2 ml of EtOH and 2 ml of pyridine. The mixture was stirred at room temperature for 2 h and concentrated to dryness. Ethyl 2-fluoro-4-nitrobenzoate was obtained as a white solid after column chromatography of the residue with ethyl acetate/hexane (1/9). This solid was then dissolved in 10 ml of ethyl acetate, and Pd/C (50 mg) was added. Hydrogenation with a hydrogen balloon converted ethyl 2-fluoro-4-nitrobenzoate into the title compound.

^1H NMR δ 7.77 (t, J = 8.4 Hz, 1H), 6.41 (dd, J_1 = 8.6, J_2 = 2.2 Hz, 1H), 6.33 (dd, J_1 = 13.0, J_2 = 2.2 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.3 (b, 2H), 1.37 (t, J = 7.1 Hz, 3H).

Methyl 4-Amino-2,6-difluorobenzoate (Compound B_1)

A solution of trifluorobenzoic acid (150 mg, 0.85 mmol, Aldrich) in 0.5 ml of $SOCl_2$ was heated

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1   under reflux for 2h. The reaction mixture was

2   cooled to room temperatur , and excess of SOCl$_2$ was

3   removed under reduced pressure. The residue was

4   dissolved in 1 ml of pyridine and 0.2 ml of

5   methanol. After stirring at room temperature for 30

6   min, solvent was removed and the residue was

7   purified by column chromatography (ethyl

8   acetate/hexane 1/10) to give methyl trifluoro-

9   benzoate as a colorless oil. This oil was then

10 dissolved in 1 ml of CH$_3$CN, then a solution of NaN$_3$

11 (100 mg, 1.54 mmol) in 0.5 ml of water was added.

12 The reaction mixture was refluxed for two days.

13 Salt was filtered and the remaining solution was

14 concentrated to an oil. This oil was then dissolved

15 in 1 ml of methanol, followed by a catalytic amount

16 of Pd/C (10%, w/w). The reaction mixture was

17 hydrogenated under a hydrogen balloon for 12 h.

18 Catalyst was removed and the solution was

19 concentrated to an oil. After column chromatography

20 (ethyl acetate/hexane 1/3), the title product was

21 obtained as colorless crystals.

22 ^{1}H NMR δ 6.17 (d, J = 10.44 Hz, 2H), 4.2 (b, 2H),

23 3.87 (s, 3H).

24 <u>8-Bromo-2,2,4,4-tetramethyl-6-chromanoic acid</u>

25 (Compound P)

26   To a solution of 2,2,4,4-tetramethyl-6-chro-

27 manoic acid (200 mg, 0.85 mmol) in 0.5 ml of AcOH

28 was added Br$_2$ (0.07 ml, 1.28 mmol). The resulting

29 dark-orange solution was stirred at room temperature

30 for overnight. The excess bromine was removed under

31 reduced pressure. Then the solution was poured into

32 5 ml of water and extracted with ethyl acetate

33 (3x3ml). The combined ethyl acetat layers were

34 further washed with NaHCO$_3$ (sat.), brine and dried

75

1   over MgSO₄. After concentration, the residue was

2   purified by column chromatography (silica gel, ethyl

3   acetate/hexane 1/3) to yield the desired product

4   (170 mg, as white solids.

5   ¹H NMR δ 8.11 (d, J = 2.2 Hz, 1H), 8.00 (d, J = 2.2

6   Hz, 1H), 1.90 (s, 2H), 1.43 (s, 6H), 1.39 (s, 6H).

7   8-Iodo-2,2,4,4-tetramethyl-6-chromanoic Acid

8   (Compound X)

9   To a solution of 2,2,4,4-tetramethyl-6-chro-

10 manoic acid (66 mg, 0.28 mmol) in 0.8 ml of AcOH was

11 added ICl (0.07 ml, 1.4 mmol). The resulting

12 colored solution was stirred at room temperature for

13 overnight. Following the same procedure as for the

14 synthesis of 8-bromo-2,2,4,4-tetramethyl-6-

15 chromanoic acid (Compound P), the reaction gave the

16 title compound (107 mg) as white solids.

17 ¹H NMR δ 8.35 (d, J = 2.2 Hz, 1H), 8.03 (d, J = 2.2

18 Hz, 1H), 1.87 (s, 2H), 1.43 (s, 6H), 1.38 (s, 6H).

19 2,2,4,4-Tetramethyl-8-trifluoromethylchroman-6-oic

20 acid (Compound S)

21   A solution of 8-bromo-2,2,4,4-tetramethyl-6-

22 chromanoic acid (Compound R, 150 mg, 0.48 mmol) in 1

23 ml of SOCl₂ was refluxed for 2 h. After cooling to

24 room temperature, the excess of SOCl₂ was removed

25 under reduced pressure and the residue was dissolved

26 in 1 ml of pyridine and 0.2 ml of methanol. The

27 mixture was stirred at room temperature for 30 min.

28 Solvent was removed and the residue was passed

29 through a column (silica gel, ethyl acetate/hexane

30 1/10) to give the methyl 8-bromo-2,2,4,4-tetra-

31 methylchromanoate (158 mg) as a colorless oil. To a

32 solution of this methyl ester in 3 ml of

33 N-m thylpyrrolidone (NMP) was added NaCO₂CF₃ (502 mg,

34 3.7 mmol) and CuI (350 mg, 1.84 mmol). The

resulting mixture was heated to 175 °C (bath temp) for 2 h. The resulting mixture was cooled to room temperature and poured into ice-water. The product was extracted into ethyl acetate (3x3ml). The combined organic layers were dried and concentrated to dryness. The crude material was purified by column chromatography (ethyl acetate/chloroform 1/10) to give the title compound as a colorless oil (120 mg). This was hydrolyzed under standard conditions to give the title compound.

^{1}H NMR δ 8.21 (d, J = 2.1 Hz, 1H), 8.17 (d, J = 2.1 Hz, 1H), 1.92 (s, 2H), 1.41 (s, 12H).

Ethyl 8-Nitro-2,2,4,4-tetramethyl-6-chromanoate (Compound W)

Ethyl 2,2,4,4-tetramethyl-6-chromanoate (150 mg, 0.57 mmol) was slowly added to 0.3 ml of conc. H_2SO_4 at 0 °C. To this mixture was added very slowly 0.03 ml of HNO_3. The reaction mixture was stirred at 0 °C for 30 min and poured into ice-water. The product was extracted into 5 ml of ethyl acetate, washed with $NaHCO_3$ (sat.), brine and dried over $MgSO_4$. After concentration, the product was purified by column chromatography (ethyl acetate/hexane 1/10) to yield 74 mg of light-yellow oil.

^{1}H NMR δ 8.24 (d, J = 2.1 Hz, 1H), 8.17 (d, J = 2.1 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.95 (s, 2H), 1.43 (s, 6H), 1.42 (s, 6H), 1.40 (t, J = 7.1 Hz, 3H).

2-Oxo-4,4,8-trimethylchroman (Compound P_1)

In a 500 ml of round bottom flask, NaH (1.66 g, 60% suspension in oil, 0.046 mol) was washed with dry hexane. Then, dry THF (22 ml) was added followed by o-cresol (5 g, 0.046 mol) in 10 ml of dry THF. The reaction mixture was stirred at 0 °C

for 30 min followed by addition of 3,3-dimethyl
acryloyl chloride in 10 ml of THF. The resulting
white slurry was stirred at room temperature for 12
h, then slowly quenched with water. The mixture was
then extracted with ethyl acetate. The organic
layer was washed with brine, water and dried over
$MgSO_4$. After filtration and removal of the solvent,
a yellow oil was obtained (10.44 g). This oil was
then dissolved in 50 ml of dry CH_2Cl_2, and was
canulated into a solution of $AlCl_3$ (10.8 g, 0.069
mmol) in 10 ml of CH_2Cl_2. The reaction mixture was
stirred at room temperature for 12 h. Then
ice-water was carefully added and the organic layer
was separated, and washed with $NaHCO_3$ (sat), brine,
water and finally dried over $MgSO_4$. After removal of
the drying agent and solvent, the residue was
purified by column chromatography (silica gel, ethyl
acetate/hexane 1/9) to yield the title compound
(4.408 g) as an oil.
^1H NMR δ 7.1 (m, 3H), 2.62 (s, 2H), 2.33 (s, 3H),
1.36 (s, 6H).

<u>2,4-Dimethyl-4-(2'-hydroxy-3'-methylphenyl)pentan-2-</u>
<u>ol</u> (Compound R_1)
     To a solution of 2-oxo-4,4,8-trimethylchroman
(Compound P_1, 2.20 g, 11.5 mmol) in 40 ml of dry
ethyl ether was added methyl magnesium bromide
(12.67 ml, 38 mmol, 3 M solution in THF). The
reaction mixture was stirred at room temperature for
12 h, then quenched with NH_4Cl (sat.) until all
precipitate dissolved. The mixture was extracted
with diethyl ether and the combined organic layers
were separated and washed with brine, water and
dried over $MgSO_4$. After filtration and removal of
the solvent, the title compound was obtained as a

1 tan solid (2.215 g).

2 ^{1}H NMR δ 7.16 (d, J = 7.88 Hz, 1H), 7.00 (d, J = 6.72

3 Hz, 1H), 6.81 (t, J = 7.6 Hz, 1H), 5.89 (b, 1H),

4 2.21 (s, 3H), 2.17 (s, 2H), 1.48 (s, 6H), 1.10 (s,

5 6H).

6 **2, 2, 4, 4, 8-Pentamethyl-6-bromochroman** (Compound

7 Z)   A solution of 2,4-dimethyl-4-(2'-hydroxy-3'-

8 methylphenyl)pentan-2-ol (Compound R_1, 2.215 g, 9.98

9 mmol) in 30 ml of 15% of H_2SO_4 was heated to 110 °C.

10 After cooling to room temperature, the reaction

11 mixture was extracted with diethyl ether. The

12 organic layer was washed with $NaHCO_3$ (sat.), brine

13 and water. After filtration and removal of solvent,

14 the residue was passed through a column (silica gel,

15 pure hexane) to give the title compound as a clear

16 oil (1.636 g). This oil was then dissolved in 1.5

17 ml of HOAc, then Br_2 (0.4113 ml, 7.98 mmol) was

18 added. The reaction mixture was stirred at room

19 temperature for 12 h. Solvent was removed under

20 reduced pressure and to the residue was added ethyl

21 acetate, and the resulting mixture was washed with

22 $NaHCO_3$ (sat.), brine, water and dried over $MgSO_4$.

23 After filtration and removal of solvent, the residue

24 was passed through a column (silica gel, pure

25 hexane) to give the title compound as a white solid

26 (2.227 g).

27 ^{1}H NMR δ 7.21 (s, 1H), 7.06 (s, 1H), 2.14 (s, 3H),

28 1.79 (s, 2H), 1.32 (s, 6H), 1.31 (s, 6H).

29 **2,2,4,4,8-Pentamethyl-6-chromanoic Acid** (Compound A_1)

30   To a solution of 2,2,4,4, 8-pentamethyl-6-bromo-

31 chroman (Compound Z) (1.2 g, 4.24 mmol) in 18 ml of

32 dry THF at -78 °C under argon gas was added slowly

33 5.48 ml of t-BuLi (1.7 M in hexane, 9.33 mmol). The

34 reaction mixture was stirr d at -78 °C for 1 h. Then

79

1 CO$_2$ was bubbled through the solution for 1 h. After

2 removal of CO$_2$ stream, the reaction mixture was

3 stirred for an additional hour at -78 °C. Then 10%

4 of HCl was added. After warming up to room

5 temperature, the reaction mixture was extracted with

6 ethyl acetate. The organic layer was further washed

7 with brine and dried over Na$_2$SO$_4$. After

8 concentration, the residue was purified by column

9 chromatography (ethyl acetate/hexane 5/95) to yield

10 the title compound as a white solid (774 mg).

11 ^{1}H NMR δ 7.96 (s, 1H), 7.75 (s, 1H), 2.23 (s, 3H),

12 1.88 (s, 2H), 1.39 (s, 6H).

13 <u>8-Bromo-4,4-dimethyl-6-chromanoic Acid</u> (Compound B$_1$)

14   Using the same procedure as for the synthesis of

15 8-bromo-2,2,4,4-tetramethylchromanoic acid (Compound

16 F) but using 4,4-dimethylchromanoic acid (100 mg,

17 0.49 mmol), the title compound was obtained as a

18 white solid.

19 ^{1}H NMR δ 8.10 (d, J = 2.1 Hz, 1H), 7.98 (d, J = 2.1

20 Hz, 1H), 4.39 (t, J = 5.44 Hz, 2H), 1.89 (t, J = 5.4

21 Hz, 1H), 1.38 (s, 6H).

22 <u>Ethyl 2-Amino-1-bromo-5,5,8,8-tetrahydro-5,5,8,8-</u>

23 <u>tetramethylnaphthalene-3-carboxylate</u> (Compound D)

24   To a solution of ethyl 5,6,7,8-tetrahydro-

25 5,5,8,8-tetramethyl-3-aminonaphthalene-2-carboxylate

26 (Compound C, 58 mg, 0.21 mmol) in 2 ml of HOAc was

27 added Br$_2$ (0.02 ml, 0.42 mmol). The orange solution

28 was stirred at room temperature for 2 days. The

29 excess Br$_2$ and HOAc were removed under reduced

30 pressure and the residue was passed through a column

31 (silica gel, ethyl acetate/hexane 1/10) to yield the

32 title compound as a light-orange oil (59 mg, 79.5%).

33 ^{1}H NMR δ 7.90 (s, 1H), 6.41 (b, 2H), 4.36 (q, J = 7.2

34 Hz, 2H), 1.70 (m, 4H), 1.58 (s, 6H), 1.40 (t, J =

7.2 Hz, 3H), 1.28 (s, 6H).

Ethyl 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl
-4-bromonaphthalene-2-carboxylate (Compound E)

Ethyl 2-Amino-1-bromo-5,5,8,8-tetrahydro-
5,5,8,8-tetramethylnaphthalene-3-carboxylate
(Compound D, 59 mg, 0.17 mmol) was dissolved in 2 ml
of EtOH at 0°C. To this solution was added 1ml of
trifluoroacetic acid and 1 ml of isoamylnitrite.
The reaction mixture was stirred at 0°C for 30 min
then H_3PO_2 (0.325 ml, 3.14 mmol) was added. The
reaction mixture was allowed to warm to room
temperature and stirred for 12 h. $NaHCO_3$ (sat.) was
added and the reaction mixture was extracted with
ethyl acetate, dried over $MgSO_4$, filtered and
concentrated to give an oil. The product was
purified by column chromatography (silica gel, ethyl
acetate/hexane 1/10) to give the title compound as a
colorless oil.

^{1}H NMR δ 8.02 (d, J = 2.0 Hz, 1H), 7.95 (d, J = 2.0
Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 1.71 (m, 4H),
1.56 (s, 6H), 1.38 (t, J = 7.1 Hz, 3H), 1.31 (s,
6H).

Ethyl
5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-fluoro-
naphthalen-2-yl-carboxylate (Compound G)

In an ice bath, ethyl
5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-aminonaphth
alene-2-carboxylate (Compound C, 150 mg, 0.55 mmol)
was added 0.24 ml of HBF_4 (48% solution in water),
followed by a solution of $NaNO_2$ (81 mg, 1.16 mmol) in
1 ml of water. The slurry was left in a
refrigerator for 3 days. The r action mixture was
washed successively with ethyl acetate until TLC
showed no UV visible spot at the baseline. The

1 thyl acetate layer was dried with MgSO₄ and th

2 solution was concentrated to an oil. The oil was

3 further dissolved in 1 ml of toluene and the mixture

4 was heated under reflux for 2 h. After the reaction

5 cooled to room temperature, solvent was evaporated

6 and the residue was passed through a column (silica

7 gel, ethyl acetate/hexane 1/10) to give the title

8 compound as an oil.

9 ^{1}H NMR δ 7.85 (d, J = 7.8 Hz, 1H), 7.04 (d, J = 12.3

10 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.69 (s, 4H),

11 1.38 (t, J = 7.1 Hz, 3H), 1.30 (s, 6H), 1.28 (s,

12 6H).

13 2-Bromo-3-hydroxy-5,5,8,8-tetrahydro-5,5,8,8-tetrame

14 thylnaphthalene (Compound I)

15   Using the same procedure as for the synthesis of

16 8-bromo-2,2,4,4-tetramethyl-6-chromanoic acid

17 (Compound P) but using 2-hydroxy-5,5,8,8-tetrahydro-

18 5,5,8,8-tetramethyltetralin (700 mg, 3.43 mmol) and

19 Br₂ (0.177 ml, 3.43 mmol) in 1.5 ml of HOAc, the

20 title compound was obtained as a white solid (747

21 mg).

22 ^{1}H NMR δ 7.36 (s, 1H), 6.96 (s, 2H), 5.32 (b, 1H),

23 1.66 (s, 4H), 1.25 (s, 12H).

24 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-methoxymet-

25 hoxy-2-bromonaphthalene (Compound J)

26   To a solution of 2-bromo-3-hydroxy-5,5,8,8-tet-

27 rahydro-5,5,8,8-tetramethylnaphthalene (Compound I,

28 600 mg, 2.12 mmol) and catalytic amount of Bu₄NBr in

29 20 ml of dry CH₂Cl₂ at 0 °C was added

30 diisoproylethylamine (1.138 ml, 12.75 mmol),

31 followed by methoxymethyl chloride (0.484 ml, 6.39

32 mmol). The reaction mixture was heated at 45 °C for

33 12 h. The reaction mixture was washed with 10% of

34 citric acid, then NaHCO₃ (sat.), brine and dried over

82

1 MgSO₄. After filtration and removal of the solvent,

2 the residue was purified by column chromatography

3 (ethyl acetate/hexane 1/9) to yield the title

4 compound (722 mg) as a white solid.

5 ¹H NMR δ 7.43 (s, 1H), 7.06 (s, 1H), 5.21 (s, 2H),

6 3.54 (s, 3H), 1.66 (s, 4H), 1.26 (s, 6H), 1.25 (s,

7 6H).

8 <u>3-Methoxymethoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrah</u>

9 <u>ydronaphthalen-2-yl carboxylic acid</u> (Compound K)

10   Using the same procedure as for the synthesis of

11 2,2,4,4,8-pentamethyl-6-chromanoic acid (Compound A₁)

12 but using 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-

13 3-methoxymethoxy-2-bromonaphthalene (Compound J, 722

14 mg, 2.21 mmol) and 2.86 ml of t-BuLi (4.87 mmol, 1.7

15 M solution in hexane), the title compound was

16 obtained as a white solid (143 mg).

17 ¹H NMR δ 8.12 (s, 1H), 7.19 (s, 1H), 5.40 (s, 2H),

18 3.58 (s, 3H), 1.70 (s, 4H), 1.30 (s, 12H).

19 <u>Ethyl 2-Fluoro-4-[(5',6',7',8'-tetrahydro-</u>

20 <u>5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]be</u>

21 <u>nzoate</u> (Compound 1)

22   To 5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-

23 2-naphthoic acid (46 mg, 0.2 mmol) was added 1 ml

24 thionyl chloride. This mixture was refluxed for 2

25 h. Excess thionyl chloride was removed under

26 reduced pressure and the residue was dissolved in 2

27 ml of CH₂Cl₂. To this solution was added ethyl

28 4-amino-2-fluorobenzoate ((Compound C₁, 37 mg, 0.2

29 mmol) followed by 0.5 ml of pyridine. The reaction

30 mixture was stirred at room temperature for 4 h and

31 was concentrated under reduced pressure. The

32 residue was purified by column chromatography (ethyl

33 acetate/hexane 1/10) to give the title compound as

34 white solids.

¹H NMR δ 8.06 (b, 1H), 7.93 (t, J = 8.4 Hz, 1H), 7.85
(d, J = 2.0 Hz, 1H), 7.78 (dd, J_1 = 2.0 Hz, J_2 = 12.9
Hz, 1H), 7.55 (dd, J_1 = 2.0 Hz, J_2 = 8.2 Hz, 1H),
7.40 (d, J = 8.3 Hz, 1H), 7.32 (dd, J_1 = 2.02 Hz, J_2
= 8.8 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 1.71 (s,
4H), 1.40 (t, J = 7.2 Hz), 1.32 (s, 6H), 1.30 (s,
6H).

Ethyl 2-Fluoro-4-[(5',6',7',8'-tetrahydro-4'-
bromo-5',5',8',8'-tetramethylnaphthalen-2'-yl)carbam
oyl]benzoate (Compound 3)

     Using the same procedure as for the synthesis of
ethyl 2-fluoro-4-[-5',6',7',8'-tetrahydro-
5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]be
nzoate (Compound 1), but using
5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-4-bromonaphth
alene-2-carboxylic acid (Compound F), the title
compound was obtained as a white solid.
¹H NMR δ 8.30 (b, 1H), 7.92 (t, J = 8.4 Hz, 1H), 7.84
(d, J = 2.1 Hz, 1H), 7.81 (d, J = 2.1 Hz, 1H), 7.74
(dd, J_1 = 2.1 Hz, J_2 = 12.8 Hz, 1H), 7.35 (dd, J_1 =
2.0 Hz, J_2 = 8.4 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H),
1.67 (m, 4H), 1.55 (s, 6H), 1.39 (t, J = 7.2 Hz,
3H), 1.31 (s, 6H).

Ethyl
2-Fluoro-4-[(3'-methoxymethoxy-5',6',7',8'-tet-
rahydro-5',
5',8',8'-tetramethylnaphthalen-2'-yl)car-
bamoyl]benzoate (Compound K_1)

     Using the same procedure as for the synthesis of
ethyl 2-fluoro-4-[(3'-methoxymethoxy-4'-bromo-
5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylnaphth
alen-2'-yl)carbamoyl]benzoate (Compound S_1), but
using 3-methoxymethoxy-5,5,8,8-tetramethyl-
5,6,7,8-tetrahydronaphthalen-2-yl carboxylic acid

84

(Compound K, 143 mg, 0.49 mmol) and

4-amino-2-fluorobenzoate (Compound C_1, 98.5 mg, 0.54

mmol), the title compound was obtained as a white

solid.

^{1}H NMR δ 10.1 (b, 1H), 8.20 (s, 1H), 7.93 (t, J = 8.8

Hz, 1H), 7.83 (d, J = 13.4 Hz, 1H), 7.29 (d, J = 8.0

Hz, 1H), 5.41 (s, 2H), 4.39 (q, J = 7.1 Hz, 2H),

3.59 (s, 3H), 1.70 (s, 4H), 1.31 (s, 12H), 1.26 (t,

J = 7.1 Hz, 3H).

<u>Ethyl 2-Fluoro-4-[(3'-hydroxy-5',6',7',8'-</u>

<u>tetrahydro-5',5',8', 8'-tetramethyl-2-</u>

<u>naphthalenyl)carbamoyl]benzoate</u>   (Compound 5)

A solution of ethyl 2-fluoro-4-[(3'-methoxymet-

hoxy-5',6',7',8'-tetrahydro-5',

5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]

benzoate (Compound K_1, 50.7 mg, 0.11 mmol) in 2 ml of

CH_2Cl_2 was added thiophenol (0.061 ml, 0.55 mmol).

The reaction mixture was stirred at 0 °C for 5 min,

then $BF_3 \cdot Et_2O$ (0.027 ml, 0.22 mmol) was added. The

reaction mixtrue was stirred at 0 °C for 2 h, then

$NaHCO_3$ (sat.) was added. The organic layer was

separated, and washed with brine, water and dried

over $MgSO_4$. After filtration and removal of solvent,

the residue was passed through a column (silica gel,

ethyl acetate/hexane 1/3) to give the title compound

as white solid (44.2 mg).

^{1}H NMR δ 8.61 (b, 1H), 7.94 (t, J = 8.42 Hz, 1H),

7.71 (dd, J = 10.8, 2.0 Hz, 1H), 7.53 (s, 1H), 7.35

(dd, J = 6.4, 2.0 Hz, 1H), 6.96 (s, 1H), 4.39 (q, J

= 7.1 Hz, 2H), 1.69 (s, 4H), 1.40 (t, J = 7.1 Hz,

3H), 1.29 (s, 6H), 1.27 (s, 6H).

<u>Ethyl 2-Fluoro-4-[(4',4'-dimethyl-8'-bromochroman-</u>

<u>6'-yl)carbamoyl]benzoate</u> (Compound 7)

In a 10 ml of round bottom flask,

85

1   4,4-dimethyl-8-bromo-6-chromanoic acid (Compound B₁,

2   139 mg, 0.485 mmol) was added SOCl₂ (1 ml, large

3   excess). The resulting solution was heated at 90 °C

4   for 2 h and allowed to cool to room temperature.

5   The excess of SOCl₂ was evaporated under reduced

6   pressure. The residue was dissolved in CH₂Cl₂ (3

7   ml). Ethyl 4-amino-2-fluorobenzoate (Compound C₁, 90

8   mg, 0.49 mmol) was added followed by pyridine (0.5

9   ml, large excess). The reaction mixture was stirred

10 for overnight and then concentrated to dryness. The

11 residue was purified by column chromatography with

12 ethyl acetate/hexane (1/5) to yield the title

13 compound as a white solid (190 mg).

14 ¹H NMR δ 7.95 (t, J = 8.31 Hz, 1H), 7.88 (b, 1H),

15 7.83 (d, J = 2.2 Hz, 1H), 7.80 (d, J = 2.2 Hz, 1H),

16 7.75 (dd, J = 12.89, 2.0 Hz, 1H), 7.30 (dd, J =

17 8.55, 2.0 Hz, 1H), 4.37 (m, 5H), 1.89 (t, J = 5.49

18 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.39 (s, 6H).

19 <u>Ethyl 2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-bromo-</u>

20 <u>chroman-6'-yl)carbamoyl]benzoate</u> (Compound 9)

21   Using the same procedure as for ethyl

22 2-fluoro-4-[(4',4'-dimethyl-8'-bromochroman-6'-yl)ca

23 rbamoyl]benzoate (Compound 7), but using

24 2,2,4,4-tetramethyl-8-bromo-6-chromanoic acid

25 (Compound P, 70 mg, 0.22 mmol) and ethyl

26 4-amino-2-fluorobenzoate (Compound C₁, 38 mg, 0.22

27 mmol), the title compound was obtained as a white

28 solid (80 mg, 76%).

29 ¹H NMR δ 8.25 (b, 1H), 7.92 (t, J = 8.4 Hz, 1H),

30 7.83 (s, 2H), 7.74 (dd, J₁ = 2.0, J₂ = 13.0 Hz, 1H),

31 7.34 (dd, J₁ = 2.0, J₂ = 8.7 Hz, 1H), 4.37 (q, J =

32 7.1 Hz, 2H), 1.88 (s, 2H), 1.41 (s, 6H), 1.39 (t, J

33 = 7.1 Hz, 3H), 1.37 (s, 6H).

34 <u>Ethyl</u>

86

1 <u>2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-trifluoromet</u>

2 <u>hylchroman-6'-yl)carbamoyl] benzoate</u> (Compound 11)

3   Using the same procedure as for ethyl

4 2-fluoro-4-[(4',4'-dimethyl-8'-bromochroman-6'-yl)ca

5 rbamoyl]benzoate (Compound 7), but using

6 2,2,4,4-tetramethyl-8-trifluoromethyl-6-chromanoic

7 acid (Compound S, 57 mg, 0.19 mmol) and ethyl

8 4-amino-2-fluorobenzoate (Compound C_1, 35 mg, 0.19

9 mmol), the title compound was obtained as white

10 solids.

11 ^{1}H NMR δ 8.06 (d, J = 2.2 Hz, 1H), 7.99 (b, 1H), 7.95

12 (t, J = 8.55 Hz, 1H), 7.81 (d, J = 2.2 Hz, 1H), 7.76

13 (dd, J = 12.8, 2.1 Hz, 1H), 7.33 (dd, J = 8.55, 1.9

14 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 1.93 (s, 2H),

15 1.41 (s, 12H), 1.40 (t, J = 7.2 Hz, 3H). <u>Ethyl</u>

16 <u>2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-amino-</u>

17 <u>chroman-6'-yl)carbamoyl]benzoate</u> (Compound N_1)

18   Using 8-nitro-2, 2, 4,

19 4-tetramethylchroman-6-carboxylic acid (Compound V)

20 and following the same procedure as for the

21 synthesis of ethyl 2-fluoro-4-[(4',4'-dimethyl-

22 8'-bromochroman-6'-yl)carbamoyl]benzoate (Compound

23 7), ethyl 2-fluoro-4-[2',2',4',4'-tetramethyl-

24 8'-nitrochroman-6'-yl)]carbamoylbenzoate was

25 obtained as a white solid. This compound (50 mg,

26 0.12 mmol) was dissolved in 2 ml of methanol. A

27 catalytic amount of Pd/C was added to the solution

28 and the solution was maintained under H_2 atmosphere

29 (hydrogen balloon) for overnight. The catalyst was

30 removed by filtration and the solvent was evaporated

31 to give the title compound as a white solid.

32 ^{1}H NMR δ 7.93 (t, J = 8.43 Hz, 1H), 7.90 (b, 1H),

33 7.73 (dd, J = 12.9, 2.0 Hz, 1H), 7.29 (dd, J = 8.43,

34 1.96 Hz, 1H), 7.23 (d, J = 2.14 Hz, 1H), 7.01 (d, J

= 2.2 Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 1.88 (s, 2H), 1.39 (s, 6H), 1.38 (t, J = 7.1 Hz, 3H), 1.37 (s, 6H).

Ethyl 2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-azidochroman-6'-yl)carbamoyl]benzoate (Compound 13)

To a solution of ethyl 2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-aminochroman-6'-yl)carbamoyl]benzoate (Compound N_1, 32 mg, 0.077 mmol) in 3 ml of EtOH was added 0.5 ml of trifluoroacetic acid (TFA) and 0.5 ml of isoamylnitrite at 0°C. The reaction was stirred for 2 h when a solution of NaN_3 (5 mg,) in 0.2 ml of water was added. The reaction mixture was allowed to warm to room temperature and stirred for overnight. The solvent was removed and the residue was purified by column chromatography (silica gel, ethyl acetate/ hexane 1/10) to give the title compound as a colorless oil.

^1H NMR δ 8.0 (b, 1H), 7.94 (t, J = 7.8 Hz, 1H), 7.73 (d, J = 12.1 Hz, 1H), 7.64 (s, 1H), 7.31 (dd, J = 8.5, 2.0 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 1.90 (s, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.45 (s, 6H), 1.40 (s, 6H).

Methyl 2,6-Difluoro-4-[(2',2',4',4'-tetramethyl-8'-trifluoromethylchroman-6'-yl)carbamoyl]benzoate (Compound 15)

Using the same procedure as for ethyl 2-fluoro-4-[(4',4'-dimethyl-8'-bromochroman-6'-yl)carbamoyl]benzoate (Compound 7), but using 2,2,4,4-tetramethyl-8-trifluoromethylchromanoic acid (Compound S, 11.2 mg, 0.037 mmol) and methyl 4-amino-2,6-difluorobenzoate (Compound B_1, 6.6 mg, 0.035 mmol), the title compound was obtained as

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1   white crystals.

2   ¹H NMR δ 8.21 (b, 1H), 8.05 (s, 1H), 7.82 (s, 1H),

3   7.36 (d, J = 10.20 Hz, 1H), 3.93 (s, 3H), 1.92 (s,

4   2H), 1.40 (s, 12H).

5   Ethyl 2-Fluoro-4-[(2', 2', 4',

6   4'-tetramethyl-8'-iodochroman-6'-yl)carbamoyl]benzoa

7   te (Compound 17)

8   Using the same procedure as for ethyl

9   2-fluoro-4-[(4',4'-dimethyl-8'-bromochroman-6'-yl)ca

10 rbamoyl]benzoate (Compound 7), but using

11 2,2,4,4-tetramethyl-8-iodochromanoic acid (Compound

12 X, 81 mg, 0.25 mmol) and ethyl 4-amino-2-

13 fluorobenzoate ((Compound C₁, 55 mg, 0.30 mmol), the

14 title compound was obtained as a white solid.

15 ¹H NMR δ 8.05 (b, 1H), 8.01 (d, J = 2.2 Hz, 1H), 7.94

16 (t, J = 8.4 Hz, 1H), 7.86 (d, J = 2.2 Hz, 1H), 7.75

17 (dd, J = 12.88, 2.1 Hz, 1H), 7.33 (dd, J = 8.8, 2.1

18 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 1.89 (s, 2H),

19 1.42 (s, 6H), 1.38 (s, 6H). Ethyl

20 2-Fluoro-4-[(2',2',4',4',8'-pentamethylchroman-

21 6'-yl)carbamoyl]benzoate (Compound 19)

22   Using the same procedure as for ethyl

23 2-fluoro-4-[(4',4'-dimethyl-8'-bromochroman-6'-yl)ca

24 rbamoyl]benzoate (Compound 9), but using

25 2,2,4,4,8-pentamethyl-6-chromanoic acid (Compound

26 A₁, 92 mg, 0.37 mmol) and ethyl

27 4-amino-2-fluorobenzoate (Compound C₁, 75 mg, 0.41

28 mmol), the title compound was obtained as a white

29 solid (100 mg).

30 ¹H NMR δ 8.31 (b, 1H), 7.90 (t, J = 8.24 Hz, 1H),

31 7.76 (dd, J = 14.29, 1.7 Hz, 1H), 7.74 (s, 1H), 7.43

32 (s, 1H), 7.35 (dd, J = 8.67, 1.7 Hz, 1H), 4.32 (q, J

33 = 7.1 Hz, 2H), 2.18 (s, 3H), 1.84 (s, 2H), 1.38 (t,

34 J = 7.1 Hz, 3H), 1.35 (s, 6H), 1.34 (s, 6H).

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Ethyl
4-[(5',6',7',8'-t trahydro-5',5',8',8'-tetramethyl-2
-naphthalenyl)thiocarbamoyl]benzoate (Compound 21)

To a solution of ethyl
4-[(5',6',7',8'-tetrahydro-5',5',8',
8'-tetramethylnaphthalen-2-yl)carbamoyl]benzoate
(Compound I₁, 61 mg, 0.16 mmol) in 2 ml of anhydrous
benzene was added Lawesson's reagent (45 mg, 0.112
mmol). The resulting yellow solution was refluxed
under N₂ for 2 h. The solvent was removed and the
residue was purified by column chromatography
(silica gel, ethyl acetate/hexane 1/5) to give the
title compound as a yellow solid (55 mg, 87%).
¹H NMR δ 9.04 (b, 1H), 8.11 (d, J = 8.70 Hz, 2H),
7.85 (b, 2H), 7.75 (b, 1H), 7.55 (dd, J = 8.2, 1.9
Hz, 1H), 7.36 (d, J = 8.3 Hz, 1H), 4.38 (q, J = 7.1
Hz, 2H), 1.71 (s, 4H), 1.40 (t, J = 7.1 Hz, 3H),
1.30 (s, 12H).

Ethyl 2-Fluoro-4-[(5',6',7',8'-tetrahydro-
5',5',8',8'-tetramethylnaphthalen-2'-yl)thiocarbamoy
l]benzoate (Compound 23)

Using the same procedure as for the synthesis of
ethyl 4-[(5',6',7',8'-tetrahydro-5',5',8',8'-
tetramethyl-2-naphthalenyl)thiocarbamoyl]benzoate
(Compound 21) but using ethyl
2-fluoro-4-[(5',6',7',8'-tetrahydro-5',5',8',8'-tetr
amethylnaphthalen-2'-yl)carbamoyl]benzoate (Compound
1, 167 mg, 0.42 mmol) in 8 ml of benzene and
Lawensson's reagent (220 mg, 0.544 mmol), the title
compound was obtained as a bright yellow solid
(127.5 mg).
¹H NMR δ 9.30 (b, 1H), 8.05 (b, 1H), 7.95 (t, J =
8.37 Hz, 1H), 7.77 (d, J = 1.89 Hz, 1H), 7.53 (dd, J
= 8.24, 2.1 Hz, 1H), 7.49 (b, 1H), 7.35 (d, J = 8.24

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1   Hz, 1H), 4.33 (q, J = 7.1 Hz, 1H), 1.71. (s, 4H),

2   1.32 (s, 6H), 1.30 (s, 6H).

3   <u>3-Hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronap</u>

4   <u>hthalen-2-yl carboxylic acid</u> (Compound L)

5   To a solution of 2-bromo-3-methoxymethoxy-

6   5,5,8,8-tetrahydro-5,5,8,8-tetramethylnaphthalene

7   (Compound J, 722 mg, 2.2 mmol) in 10 ml of dry THF

8   at -78°C under argon was added slowly 2.86 ml of

9   t-BuLi (1.7 M in hexane, 4.8 mmol). The reaction

10   mixture was stirred at -78°C for 1 h. Then CO_2 was

11   bubbled through the solution for 1 h. After removal

12   of CO_2 stream, the reaction mixture was stirred for

13   an additional hour at -78°C. Then 10% of HCl was

14   added. After warming up to room temperature, the

15   reaction mixture was left overnight then extracted

16   with ethyl acetate. The organic layer was washed

17   with brine and dried over Na_2SO_4. After

18   concentration, the residue was purified by column

19   chromatography (ethyl acetate/hexane 1/3) to yield

20   the title compound as a white solid.

21   ^{1}H NMR d 7.85 (s, 1H), 6.93 (s, 1H), 1.68 (s, 4H),

22   1.28 (s, 12H).

23   <u>4-Bromo-3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrah</u>

24   <u>ydronaphthalen-2-yl carboxylic acid</u> (Compound M)

25   3-Hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-

26   naphthalen-2-yl acid (Compound L, 155 mg, 0.62 mmol)

27   was dissolved in 1 ml of HOAc. To this solution was

28   added Br_2 (0.033 ml, 0.62 mmol). The reaction

29   mixture was left at room temperature for over night.

30   A stream of air was passed through the reaction

31   mixture to remove the unreacted Br_2. The remaining

32   solid was dissolved in small amount of THF and

33   purified by column chromatography (ethyl

34   acetate/hexane 1/1) to yield the desired product as

a cream colored solid.

¹H NMR d 7.91 (s, 1H), 1.75 (m, 2H), 1.64 (m, 2H),

1,62 (s, 6H), 1.30 (s, 6H).

<u>4-Bromo-3-methoxymethoxy-5,5,8,8-tetramethyl-5,6,7,8</u>
<u>-tetrahydronaphthalen-2-yl carboxylic acid</u> (Compound
N)

To a solution of 4-bromo-3-hydroxy-5,5,8,8-
tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl acid
(Compound M), 233 mg, 0.71 mmol) in 6 ml of CH_2Cl_2
was added chloromethyl methyl ether (0.162 ml, 2.1
mmol), diisopropylethyl amine (0.764 ml, 4.2 mmol)
and a catalytic amount of tetrabutylammouimn
bromide. The reaction mixture was heated to 45 °C
for 2 h. The reaction mixture was concentrated and
the residue was purified by column chromatography
(ethyl acetate/hexane 1/9) to yield the
methoxymethyl ester of the title compound as a white
solid (200 mg). This white solid was further
dissolved in 20 ml of EtOH. An aqueous solution of
NaOH (0.5 ml, 1M) was added. The reaction mixture
was stirred at room temperature for over night. The
EtOH was removed and the residue was added 2 ml of
ethyl acetate and 3 ml of water. This mixture was
very slowly acidified with 10% HCl to PH = 7. The
ethyl acetate layer was separated and washed with
brine, dried over Na_2SO_4. After filtration of the
drying agent and removal of solvent, the reaction
yielded the title compound as a white solid (155
mg). ¹H NMR d 7.99 (s, 1H), 5.20 (s, 2H), 3.66 (s,
3H), 1.74 (m, 2H), 1.67 (m, 2H), 1.60 (s, 6H), 1.32
(s, 6H).

<u>Ethyl 2-fluoro-4-[(3'-methoxymethoxy-4'-bromo-</u>
<u>5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylnaphth</u>
<u>alen-2'-yl)carbamoyl]benzoate</u> (Compound S_1)

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To a solution of 4-bromo-3-methoxymethoxy-
5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-y
l acid (Compound N, 80 mg, 0.22 mmol) in 4 ml of
CH_2Cl_2 was added DMAP (60 mg, 0.26 mmol), ethyl
2-fluoro-4-aminobenzoate (Compound C_1, 43 mg, 0.24
mmol) and EDC (50 mg, 0.26 mmol). The reaction
mixture was stirred at room temperature for
overnight and then concentrated to dryness. The
residue was purified by column chromatography (ethyl
acetate/hexane 1/3) to yield the title compound as a
clear oil (45 mg).
^{1}H NMR d 9.92 (b, 1H), 8.10 (s, 1H), 7.94 (t, J = 8.4
Hz, 1H), 7.81 (dd, J = 12.9; 1.9 Hz, 1H), 7.35 (dd,
J = 8.5; 1.8 Hz, 1H), 5.20 (s, 2H), 4.39 (q, J =
7.1 Hz, 2H), 3.61 (s, 3H), 1.74 (m, 2H), 1.64 (m,
2H), 1.60 (s, 6H), 1.40 (t, J = 7.1 Hz, 3H), 1.34
(s, 6H).

Methyl
2,6-Difluoro-4-[(3'-methoxymethoxy-4'-bromo-5',6',7'
,8'-tetrahydro-5',5',8',8'-tetramethylnaphtha-
len-2'-yl)carbamoyl]benzoate (Compound M_1)

Using the same procedure as for the synthesis of
compound ethyl 2-fluoro-4-[(3'-methoxymethoxy-4'-
bromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl
naphthalen-2'-yl)carbamoyl]benzoate (Compound S_1) but
using 4-bromo-3-methoxymethoxy-5,5,8,8-tetramethyl-
5,6,7,8- tetrahydronaphthalen-2-yl acid (Compound N,
80 mg, 0.22 mmol), DMAP (60 mg, 0.26 mmol), methyl
2,6-difluoro-4-aminobenzoate (Compound E_1, 52 mg,
0.24 mmol) and EDC (50 mg, 0.26 mmol), the title
compound was obtained as a clear oil.
^{1}H NMR d 10.01 (b, 1H), 8.11 (s, 1H), 7.42 (d, J =
10.0 Hz, 2H), 5.2 (s, 2H), 3.95 (s, 3H), 3.63 (s,
3H), 1.75 (m, 2H), 1.65 (m, 2H), 1.61 (s, 6H), 1.35

1 (s, 6H).

2 4-Bromomethyl-2,6-di-t-butylpyridine (Compound A₃)

3   To a mixture of 2,6-di-t-butyl-4-methylpyridine

4 (Aldrich, 2.0 g, 9.73 mmol) in 25 ml of dry CCl₄ was

5 added benzoyl peroxide (24 mg, 0.097 mmol) and NBS

6 (1.9 g, 10.7 mmol). The reaction mixture was

7 refluxed for 16 hours. After it cooled to room

8 temperature, the solvent was removed in vacuo and

9 the residue was purified by column chromatography

10 (silica gel, hexane) to give an oil (1.957 g) which

11 contained 82% of the desired product and 18% of the

12 starting material. ¹H NMR δ 7.09 (s, 2H), 4.39 (s,

13 2H), 1.35 (s, 18H).

14 4-Hydroxymethyl-2,6-di-t-butylpyridine (Compound B₃)

15   A heterogeneous solution of

16 4-bromomethyl-2,6-di-t-butylpyridine (Compound A₃,

17 1.743 g, 82% purity) in 20 ml of 12% NaOH in water

18 and 10 ml of 1,4-dioxane was refluxed for 12 hours.

19 The solution spontaneously separated into two layers

20 as it cooled to room temperature. The upper layer

21 was separated and ethyl acetate was added. This

22 organic layer was then washed with brine, water and

23 dried over MgSO₄. The desired product was purified

24 by column chromatography (ethyl acetate/hexane 1/9)

25 to give a white solid. ¹H NMR δ 7.09 (s, 2H), 4.67

26 (d, J = 4.4 Hz, 2H), 2.3 (b, 1H), 1.36 (s, 18H).

27 2,6-Di-t-butylisonicotinic acid (Compound C₃)

28 Jone's reagent was added dropwise to a solution of

29 4-hydroxymethyl-2,6-di-t-butylpyridine (Compound B₃,

30 302 mg, 1.37 mmol) in 5 ml of acetone until the

31 solution changed color from light yellow to orange

32 (55 drops of Jone's reagent were consumed). After 5

33 minutes 2 ml of isopropanol were added to the

34 reaction mixture, and a green precipitate of Cr^{3+}

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salt was formed. The precipitate was removed by
filtration and the solution was diluted with ethyl
acetate, then washed with brine, water and dried
over MgSO₄. After filtration, the solvent was
removed to give the desired product as a white solid
(227 mg). ¹H NMR δ 7.71 (s, 2H), 1.34 (s, 18H).

2-Bromo-4,6-di-t-butylphenol (Compound D₃)

To a solution of 2,4-di-t-butylphenol (Aldrich,
2.0 g, 9.7 mmol) in 2 ml of HOAc was added Br₂ (0.5
ml, 9.7 mmol). The reaction mixture was stirred at
room temperature for 12 hours. Solvent was removed
under reduced pressure and the residue was purified
by column chromatography (ethyl acetate/hexane 1/20)
to yield the desired product (2.54 g) as a white
solid. ¹H NMR δ 7.33 (d, J = 2.3 Hz, 1H), 7.24 (d, J
= 2.3 Hz, 1H), 1.41 (s, 9H), 1.29 (s, 9H).

O-Methoxymethyl-2-bromo-4,6-di-t-butylphenol
(Compound E₃)

To a solution of 2-bromo-4,6-di-t-butylphenol
(Compound D₃ 2.54 g, 8.88 mmol) and catalytic amount
of Bu₄NI in 20 ml of dry CH₂Cl₂ at 0°C was added
diisopropylethylamine (9.51 ml, 53 mmol), followed
by methoxymethyl chloride (2.02 ml, 26.6 mmol). The
reaction mixture was heated to 45°C for 12 hours.
The reaction mixture was then washed with 10% citric
acid, then NaHCO₃ (sat.), brine, and dried over
MgSO₄. After filtration and removal of the solvent
under reduced pressure, the residue was purified by
column chromatography (pure hexane) to yield the
title compound (2.79 g) as a colorless oil. ¹H NMR δ
7.40 (d, J = 2.44 Hz, 1H), 7.30 (d, J = 2.4 Hz, 1H),
5.22 (s, 2H), 3.70 (s, 3H), 1.43 (s, 9H), 1.29 (s,
9H).

O-Methoxymethyl-3',5'-di-t-butylsalicylic acid

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1   (Compound F$_3$)

2   To a solution of O-methoxymethyl-2-bromo-4,6-

3   di-t-butylphenol (Compound E$_3$, 2.79 g, 8.5 mmol) in

4   30 ml of dry THF at -78°C under Ar was added 11 ml

5   of t-BuLi (1.7 M in hexane, 18.7 mmol). This

6   mixture was stirred at -78°C for 1 hour. Then CO$_2$

7   (g) was bubbled into the solution at -78°C for 1

8   hour. After removal of the CO$_2$ stream, the reaction

9   mixture was stirred for an additional hour at -78°C.

10   Then 10% of HCl was added and the mixture was

11   allowed to warm to room temperature and extracted

12   with ethyl acetate. The organic layer was washed

13   with brine and dried over Na$_2$SO$_4$. After

14   concentration, the residue was purified by column

15   chromatography (ethyl acetate/hexane 1/1) to yield

16   the title compound as a white solid (492 mg). ^{1}H NMR

17   δ 7.75 (d, J = 2.81 Hz, 1H), 7.60 (d, J = 2.8 Hz,

18   1H), 5.07 (s, 2H), 3.62 (s, 3H), 1.33 (s, 9H), 1.26

19   (s, 9H).

20   Ethyl 2-fluoro-4-[(2'6'-di-t-butylpyrid-4'-

21   yl)carbamoyl]benzoate (Compound 41)

22   A solution of 2,6-di-t-butylisonicotinic acid

23   (Compound C$_3$, 47.3 mg, 0.20 mmol) in 2 ml of SOCl$_2$

24   was heated under reflux for 2 hours. Excess SOCl$_2$

25   was removed in vacuo and the residue was dissolved

26   in 2 ml of dry CH$_2$Cl$_2$, and ethyl

27   2-fluoro-4-aminobenzoate (Compound C$_1$, 40.2 mg, 0.22

28   mmol) and pyridine (0.0835 ml, 0.69 mmol) were

29   added. The reaction mixture was stirred at room

30   temperature for 12 hours. Solvent was removed and

31   the residue was purified by column chromatography

32   (ethyl acetate/hexan 1/9) to yield the title

33   compound (71.2 mg) as white crystals. ^{1}H NMR δ 8.56

34   (b, 1H), 7.91 (t, J = 8.36 Hz, 1H), 7.53 (dd, J =

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1   12.82, 2.0 Hz, 1H), 7.39 (dd, J = 8.7, 2.0 Hz, 1H),

2   4.33 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H),

3   1.35 (s, 18H).

4   Ethyl 4-[(2',6'-di-t-butylpyrid-4'-yl)car-

5   bamoyl]benzoate (Compound 43)

6   Using the same procedure as for the synthesis of

7   ethyl 2-fluoro-4-[(2'6'-di-t-butylpyrid-4'-

8   yl)carbamoyl]benzoate (Compound 41) but using

9   2,6-di-t-butylisonicotinic acid (Compound C_3, 101 mg,

10   0.43 mmol) and ethyl 4-aminobenzoate (78 mg, 0.47

11   mmol), the title compound was obtained as a white

12   solid (135 mg). ^{1}H NMR δ 8.43 (b, 1H),, 8.02 (d, J =

13   8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.48 (s, 2H),

14   4.33 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H),

15   1.35 (s, 18H).

16   Ethyl

17   2-Fluoro-4-[(3',5'-di-t-butylphenyl)carbamoyl]benzoa

18   te (Compound 45)

19   Using the same procedure as for the synthesis of

20   ethyl 2-fluoro-4-[(2'6'-di-t-butylpyrid-4'-

21   yl)carbamoyl]benzoate (Compound 41) but using

22   3,5-di-t-butylbenzoic acid (60 mg, 0.26 mmol,

23   available by literature procedure, see Kagechika et

24   al. J. Med Chem. 1988 31, 2182 - 2192) and ethyl

25   2-fluoro-4-aminobenzoate (Compound C_1, 51.5 mg, 0.28

26   mmol), the title compound was obtained as a white

27   solid (66 mg). ^{1}H NMR δ 8.21 (b, 1H), 7.93 (t, J =

28   8.3 Hz, 1H), 7.79 (dd, J = 12.8, 2.0 Hz, 1H), 7.67

29   (d, J = 1.8 Hz, 2H), 7.65 (t, J = 1.7 Hz, 1H), 7.35

30   (dd, J = 8.7, 2.1 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H),

31   1.39 (t, J = 7.2 Hz, 3H), 1.36 (s, 18H).

32   Ethyl

33   2-Fluoro-4-[(2'-methoxym thyl-3',5'-di-t-butylphenyl

34   )carbamoyl]benzoate (Compound G_3)

1   To a mixtur of O-methoxymethyl-3',5'-di-t-

2   butylsalicylic acid (Compound F₃, 150 mg, 0.51 mmol),

3   4-dimethylaminopyridine (142 mg, 0.61 mmol) and

4   ethyl 2-fluoro-4-aminobenzoate (Compound C₁, 102 mg,

5   0.56 mmol) in 5 ml of dry CH₂Cl₂ was added 1-(3-di-

6   methylaminopropyl)-3-ethylcarbodiimide hydrochloride

7   (117 mg, 0.61 mmol). The reaction mixture was

8   stirred at room temperature for 12 hours. Solvent

9   was evaporated in vacuo and the residue was

10   dissolved in ethyl acetate, then washed with brine,

11   water and dried over MgSO₄. After filtration,

12   solvent was removed and the residue was purified by

13   column chromatography (ethyl acetate/hexane 1/3) to

14   give the title compound (58 mg). ¹H NMR δ 8.97 (b,

15   1H), 7.94 (t, J = 8.37 Hz, 1H), 7.78 (d, J = 2.7 Hz,

16   1H), 7.61 (d, J = 13.0 Hz, 1H), 7.56 (d, J = 2.6 Hz,

17   1H), 7.35 (d, J = 8.7 Hz, 1H), 5.00 (s, 2H), 3.53

18   (s, 3H), 4.38 (q, J = 7.1 Hz, 2H), 1.47 (s, 9H),

19   1.39 (t, J = 7.2 Hz, 3H), 1.33 (s, 9H).

20   Ethyl

21   2-Fluoro-4-[(2'-hydroxy-3',5'-di-t-butylphenyl)carba

22   moyl]benzoate (Compound 47)

23   To a solution of ethyl 2-fluoro-4-[(2'-

24   methoxymethyl-3',5'-di-t-butylphenyl)carbamoyl]benzo

25   ate (Compound G₃, 34 mg, 0.07 mmol) in 1 ml of THF

26   were added 10 drops of HOAc. The reaction mixture

27   was heated to reflux for 12 hours. Solvent was

28   removed and ethyl acetate was added. The solution

29   was washed with NaCHO₃ (sat.), brine, water and dried

30   over MgSO₄. Solvent was removed in vacuo to give an

31   oil. The oil was allowed to be exposed to the

32   atmosph re for 12 hours during which time crystals

33   formed. The crystals were coll cted and wash d

34   several times with hexane to afford the title

compound as a white solid (13.5 mg). ^{1}H NMR δ 10.73
(s, 1H), 7.98 (d, J = 2.56 Hz, 1H), 7.88 (b, 1H),
7.75 (t, J = 8.26 Hz, 1H), 7.60 (d, J = 2.44 Hz,
1H), 7.32 (dd, J = 12.3, 2.0 Hz, 1H), 7.02 (dd, J =
8.6, 2.0 Hz, 1H), 4.35 (q, J = 7.2 Hz, 2H), 1.39 (s,
9H), 1.37 (t, J = 7.2 Hz, 3H), 1.5 (s, 9H).

2,6-Difluoro-4-[(2',6'-di-t-butylpyrid-4'yl)carbamoy
l]benzoic Acid (Compound 50)

　　　To 2,6-di-t-butylisonicotinic acid (Compound C$_3$,
20 mg, 0.085 mmol) was added 1 ml of SOCl$_2$. The
mixture was heated under reflux for 2 hours. After
cooling to room temperature, excess SOCl$_2$ was removed
and the residue was dissolved in 2 ml of CH$_2$Cl$_2$. To
this solution was added methyl 2,6-difluoro-4-amino-
benzoate (Compound B$_1$, 16 mg, 0.085 mmol) and
triethylamine (0.015 ml, 0.1 mmol). The reaction
mixture was kept at room temperature for 2 hours and
then concentrated to dryness. The residue was
purified by column chromatography with ethyl
acetate/hexane (1/10) to yield the methyl ester of
the title compound. This was saponified according
to the general procedure (see below) to give the
title compound as a colorless solid. ^{1}H NMR δ 7.44
(s, 2H), 7.40 (d, J = 11.8 Hz, 2H) 1.37 (s, 18H).

2,6-Difluoro-4-[(3',5'-di-t-butylphenyl)car-bamoyl]b
enzoic Acid (Compound 52)

　　　Using the same procedure as for the preparation
of 2,6-difluoro-4-[(2',6'-di-t-butylpyrid-
4'yl)carbamoyl]benzoic acid (Compound 50) but using
3,5-di-t-butylbenzoic acid (37 mg, 0.16 mmol) and
methyl 2,6-difluoro-4-aminobenzoate (Compound B$_1$, 29
mg, 0.16 mmol), the title compound was obtained as
colorless crystals. ^{1}H NMR δ 7.92 (b, 1H) 7.60 (m,
3H), 7.42 (d, J = 10.0 Hz, 2H), 1.38 (s, 18H).

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2-Nitro-4-[(2',6'-di-t-butylpyrid-4'-yl)carbamoyl]be nzoic Acid (Compound 54)

Using the same procedure as for the preparation of 2,6-difluoro-4-[(2',6'-di-t-butylpyrid-4'yl)carbamoyl]benzoic acid (Compound 50) but using 2,6-di-t-butylisonicotinic acid (40 mg, 0.17 mmol) and methyl 2-nitro-4-aminobenzoate (Compound F_1, 33 mg, 0.17 mmol), the title compound was obtained as a light yellow oil. ^{1}H NMR δ (acetone-d^6) 10.25 (b, 1H), 8.32 (s, 1H), 7.97 (d, J = 8.1 Hz, 1H), 7.93 (b, 1H), 7.70 (s, 2H), 1.36 (s, 18H).

Methyl 2-nitro-4-[(4'-bromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]be nzoate (Compound 25)

Using the same procedure as for the synthesis of Compound 1, but using Compound F and Compound F_1, the desired product was obtained as a white solid. ^{1}H NMR δ 9.24 (b, 1H), 9.23 (d, J = 1.8 Hz, 1H), 7.92 (dd, J = 8.4, 2.4, Hz, 1H), 7.87 (d, J = 2.1 Hz, 1H), 7.84 (d, 3 = 2.1 Hz, 1H), 7.80 (d, J = 8.7 Hz, 1H), 3.91 (s, 3H), 1.75 (m, 2H), 1.65 (m, 2H), 1.58 (s, 3H), 1.33 (s, 3H).

General procedure for the syntheses of benzoic acid derivatives by hydrolyzing the corresponding methyl or ethyl esters.

To a solution of ester (3.0 mmol) in 20 ml of EtOH was added 5 ml of 1 N NaOH in water. The reaction mixture was stirred at room temperature for overnight and neutralized with 10% HCl to PH=5. The alcohol was removed by evaporation and the aqueous layer was extracted with ethyl acetate (3x10ml). The combined ethyl acetate layers were washed with NaHCO$_3$ (sat.), brine and dried over MgSO$_4$. Aft r concentration, the desired acid was obtained which

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1   could be recrystallized in ethyl acetate or in

2   acetonitrile.

3   2-Fluoro-4-[(5',6',7',8'-tetrahydro-5',5',8',8'-tetr

4   amethylnaphthalen-2'-yl)carbamoyl]benzoic Acid

5   (Compound 2)

6   ^{1}H NMR δ (acetone-D_6) 9.86 (b, 1H), 7.95 (m, 3H),

7   7.75 (dd, J = 7.9, 2.2 Hz, 1H), 7.62 (dd, J = 8.5,

8   1.6 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H), 1.73 (s, 4H),

9   1.32 (s, 6H), 1.30 (s, 6H).

10   2-Fluoro-4-[(4'-bromo-5',6',7',8'-tetrahydro-5',5',8

11   ',8'-tetramethylnaphthalen-2'-yl)carbamoyl]benzoic

12   Acid (Compound 4)

13   ^{1}H NMR δ (acetone-D_6) 9.97 (b, 1H), 8.04 (d, J = 1.89

14   Hz, 1H), 8.01 (d, J = 1.90 Hz, 1H), 7.95 (t, J =

15   8.55 Hz, 1H), 7.90 (dd, J = 12.28, 2.0 Hz, 1H), 7.59

16   (dd, J = 8.67, 1.50 Hz, 1H), 1.76 (m, 4H), 1.58 (s,

17   6H), 1.35 (s, 6H).

18   2-Fluoro-4-[(3'-hydroxy-5',6',7',8'-tetrahydro-5',5'

19   ,8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]benzoic

20   Acid (Compound 6)

21   ^{1}H NMR (acetone-D_6) δ 11.3 (b, 1H), 10.2 (b, 1H),

22   7.94 (m. 2H), 7.85 (dd, J = 11.4, 1.95 Hz, 1H), 7.53

23   (dd, J = 6.59, 2.08 Hz, 1H), 6.94 (s, 1H), 2.85 (b,

24   1H), 1.70 (s, 4H), 1.29 (s, 6H), 1.28 (s, 12H).

25   2-Fluoro-4-[(8'-bromo-4',4'-dimethylchroman-6'-yl)ca

26   rbamoyl]benzoic Acid (Compound 8)

27   ^{1}H NMR (acetone-d_6) δ 9.87 (b, 1H), 8.04 (d, J = 2.1

28   Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H), 7.94 (t, J = 8.66

29   Hz, 1H), 7.91 (dd, J = 13.8, 2.0 Hz, 1H), 7.57 (dd,

30   J = 8.6, 2.0 Hz, 1H), 4.37 (t, J = 5.44 Hz, 2H),

31   1.92 (t, J = 5.44 Hz, 2H), 1.40 (s, 6H).

32   2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-bromochroman

33   - 6'-yl)carbamoyl]benzoic Acid (Compound 10)

34   ^{1}H NMR δ (acetone-d_6) 9.87 (b, 1H), 8.06 (d, J = 2.2

Hz, 1H), 8.04 (d, J = 2.1 Hz, 1H), 7.94 (t, J = 8.54

Hz, 1H), 7.91 (dd, J = 14.0, 2.0 Hz, 1H), 7.59 (dd,

J = 8.5, 2.3 Hz, 1H), 1.96 (s, 2H), 1.42 (s, 6H),

1.41 (s, 6H).

<u>2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-trifluoro-</u>

<u>methylchroman-6'-yl)carbamoyl] benzoic Acid</u>

(Compound 12)

^{1}H NMR (acetone-d$_6$) δ 10.02 (b, 1H), 8.31 (s, 1H),

8.09 (s, 1H), 7.92 (m, 2H), 7.56 (d, J = 7.69 Hz,

1H), 2.00 (s, 2H), 1.44 (s, 6H), 1.41 (s, 6H).

<u>2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-azidochroman</u>

<u>- 6'-yl)carbamoyl]benzoic Acid</u> (Compound 14)

^{1}H NMR δ 8.03 (t, J = 8.4 Hz, 1H), 7.87 (b, 1H), 7.79

(dd, J = 13, 2.0 Hz, 1H), 7.64 (d, J = 2.2 Hz, 1H),

7.32 (dd, J = 8.66, 1.9 Hz, 1H), 7.22 (d, J = 2.1

Hz, 1H), 1.91 (s, 2H), 1.45 (s, 6H), 1.41 (s, 6H).

<u>2, 6-Difluoro-4-[(2',2',4',4'-tetramethyl-8'-</u>

<u>trifluoromethylchroman-6'-yl)carbamoyl]benzoic acid</u>

(Compound 16)

^{1}H NMR (acetone-d$_6$) δ 8.30 (d, J = 2.3 Hz, 1H), 8.06

(d, J = 2.2 Hz, 1H), 7.59 (d, J = 10.32 Hz, 2H),

1.954 (s, 2H), 1.44 (s, 6H), 1.41 (s, 6H).

<u>2-Fluoro-4-[(2',2',4',4'-tetramethyl-8'-iodochroman-</u>

<u>6'-yl)carbamoyl]benzoic Acid</u> (Compound 18)

^{1}H NMR δ (acetone-d$_6$) 10.0 (b, 1H), 8.24 (s, 1H),

8.07 (s, 1H), 7.94 (m, 2H), 7.57 (d, J = 8.67 Hz,

1H), 1.95 (s, 2H), 1.41 (s, 12H).

<u>2-Fluoro-4-[(2',2',4',4',8'-pentamethylchroman-6'-yl</u>

<u>)carbamoyl]benzoic Acid</u> (Compound 20) ^{1}H NMR δ

(acetone-d$_6$) 9.77 (b, 1H), 7.90 (m, 3H), 7.65 (d, J =

2.0 Hz, 1H), 7.56 (dd, J = 8.61, 2.0 Hz, 1H), 2.19

(s, 3H), 1.90 (s, 2H), 1.38 (s, 6H), 1.37 (s, 6H).

<u>4-[(5',6',7',8'-tetrahydro-5',5',8',8'-tetram thylna</u>

<u>phthalen-2'-yl)thiocarbamoyl]benzoic Acid</u> (Compound

22)

^{1}H NMR δ 9.08 (b, 1H), 8.17 (d, J = 8.61, 2H), 7.95 (b, 2H), 7.77 (b, 1H), 7.57 (dd, J = 8.1, 2.1 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 1.72 (s, 4H), 1.32 (s, 6H), 1.31 (s, 6H).

2-Fluoro-4-[(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylnaphthalen-2'-yl)thiocarbamoyl]benzoic Acid (Compound 24)

^{1}H NMR δ (acetone-d$_6$) 11.1 (b, 1H), 8.27 (b, J = 13.2 Hz, 1H), 8.02 (t, J = 8.3 Hz, 1H), 7.89 (s, 1H), 7.86 (d, J = 10.0 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.41 (d, J = 8.37 Hz, 1H), 1.72 (s, 4H), 1.30 (s, 12H).

2-Fluoro-4-[(3'-hydroxy-4'-bromo-5',6',7',8'-tetrahydro-5',5', 8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]benzoic Acid (Compound 30)

A solution of ethyl 2-fluoro-4-[(3'-methoxymet-hoxy-4'-bromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]benzoate (Compound S$_1$, 45 mg, 0.084 mmol) in 1 ml of EtOH was added 1 ml of aqueous solution of NaOH (1M). The reaction mixture was stirred at room temperature for overnight and acidified to PH = 1 with 10% HCl. EtOH was removed and ethyl acetate and more water were added to the solution. The organic layer was separated and washed with NaHCO$_3$, brine and dried over MgSO$_4$. After filtration and concentration, the reaction yielded 2-fluoro-4-[(3'-methoxymethoxy-4'-bromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]benzoic acid as a white solid. The methoxymethyl group was removed by dissolving the white solid in 2 ml of MeOH and 3 drops of HCl (con.). After stirring for overnight, the reaction mixtur was concentrated to dryness.

Th residue was partitioned between ethyl acetate
and water. The organic lay r was separated, washed
with NaHCO₃, brine and dried over MgSO₄. After
filtration and concentration, the residual solid was
purified in a mini (pipette) column with ethyl
acetate /hexane (1/1) to give the title compound as
a white solid (5.0 mg).

¹H NMR d (acetone-d⁶) 10.19 (b, 1H), 8.01 (s, 1H),
7.96 (t, J = 8.6 Hz, 1H), 7.76 (dd, J = 11.2; 2.0
Hz, 1H), 7.54 (dd, J = 8.8; 2.0 Hz, 1H), 1.75 (m,
2H), 1.65 (m, 2H), 1.61 (s, 6H), 1.32 (s, 6H).

<u>2,6-Difluoro-4-[(3'-hydroxy-4'-bromo-5',6',7',8'-tet</u>
<u>rahydro-5',5',8',8'-tetramethylnaphthalen-2'-</u>
<u>yl)carbamoyl]benzoic Acid</u> (Compound 32)

Using the same procedure as for the synthesis of
2-fluoro-4-[(3'-hydroxy-4'-bromo-5',6',7',8'-tetrahy
-dro-5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamo
yl]benzoic acid (Compound 30) the title compound was
obtained as a white solid.

¹H NMR d(acetone-d⁶) 10.23 (b, 1H), 8.01 (s, 1H),
7.52 (d, J = 10.2 Hz, 2H), 4.8 (b, 1H), 1.75 (m,
2H), 1.65 (m, 2H), 1.60 (s, 6H), 1.31 (s, 6H).

<u>2,6-Difluoro-4-[(5',6',7',8'-tetrahydro-5',5',8',8'-</u>
<u>tetramethylnaphthalen-2'-yl)carbamoyl]benzoic Acid</u>
(Compound 34)

To 5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
2-naphthoic acid (43 mg, 0.19 mmol) was added 1 ml
of thionyl chloride. This mixture was refluxed for
2 h. Excess thionyl chloride was removed under
reduced pressure and the residue was dissolved in 2
ml of CH₂Cl₂. To this solution was added methyl
4-amino-2,6-difluorobenzoate (Compound B₁, 7 mg, 0.2
mmol) followed by 0.5 ml of pyridine. The reaction
mixture was stirred at room temperature for 4 h and

was concentrated under reduced pressure. The
residue was purified by column chromatography (ethyl
acetate/hexane 1/5) to give the methyl ester of the
desired product as a colorless oil.

^{1}H NMR d 8.11 (d, J = 1.9 Hz, 1H), 8.05 (b, 1H), 7.86
(dd, J = 6.2, 2.2 Hz, 1H), 7.41 (m, 3H), 3.93 (s,
3H), 1.69 (s, 4H), 1.29 (s, 6H), 1.28 (s, 6H). This
colorless oil was hydrolyzed to the desired product
with NaOH/H$_2$O/EtOH according to the general
procedure.

^{1}H NMR d (acetone-d^6) 9.74 (b, 1H), 7.95 (s, 1H),
7.70 (d, J = 6.8 Hz, 1H), 7.43 (d, J = 8.4 Hz, 3H),
1.71 (s, 4H), 1.29 (s, 6H), 1.28 (s, 6H).

2-Nitro-4-[(4'-bromo-5',6',7',8'-tetrahydro-5',5',8'
,8',-tetramethylnaphthalen-2'-yl)carbamoyl]benzoic
acid (Compound 26)

^{1}H NMR δ (acetone-d^6): 10.16 (b, 1H), 8.42 (d, J =
2.0 Hz, 1H), 8.09 (dd, J = 8.6; 2.1 Hz, 1H), 8.06
(d, J = 2.2 Hz, 1H), 8.04 (d, J = 2.2 Hz, 1H), 7.93
(d, J = 8.6 Hz, 1H), 1.75 (m, 2H), 1.65 (m, 2H),
1.57 (s, 3H), 1.34 (s, 3H).

2-Fluoro-4-[(2',6'-di-t-butylpyrid-4'-yl)carbamoyl]b
enzoic Acid (Compound 42)

     ^{1}H NMR δ (CD$_3$OD) 7.92 (t, J = 8.36 Hz, 1H), 7.82
(dd, J = 12.82, 2.0 Hz, 1H), 7.63 (s, 2H), 7.55 (dd,
J = 8.7, 2.1 Hz, 1H), 1.39 (s, 18H).

4-[(2',6'-Di-t-butylpyrid-4'-yl)carbamoyl]benzoic
acid (Compound 44)

     ^{1}H NMR δ (CD$_3$OD) 8.02 (d, J = 8.85 Hz, 2H), 7.85
(d, J = 8.85 Hz, 2H), 7.63 (s, 2H), 1.40 (s, 18H).

2-Fluoro-4-[(3',5'-di-t-butyl)phenylcarbamoyl]benzoi
c acid (Compound 46)

     ^{1}H NMR δ (CD$_3$OD) 7.92 (t, J = 8.3 Hz, 1H), 7.80
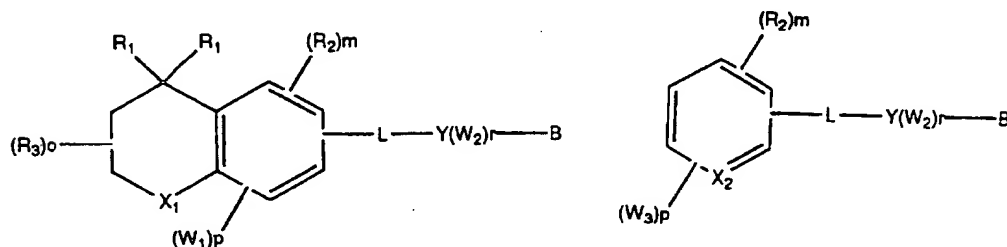(dd, J = 12.8, 2.0 Hz, 1H), 7.79 (d, J = 1.8 Hz,

2H), 7.69 (t, J = 1.7 Hz, 1H), 7.57 (dd, J = 8.7,

2.1 Hz, 1H), 1.37 (s, 18H).

<u>2-Fluoro-4-[(2'-hydroxy-3',5'-di-t-butyl)phenylcarba</u>

<u>moyl]benzoic acid (Compound 48)</u>

^{1}H NMR δ (acetone-d$_6$) 12.3 (b, 1H), 10.07 (b,

1H), 7.98 (t, J = 8.48 Hz, 1H), 7.80 (m, 2H), 7.58

(d, J = 2.3 Hz, 1H), 7.56 (dd, J = 8.8, 2.0 Hz, 1H),

1.44 (s, 9H), 1.31 (s, 9H).

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WHAT IS CLAIMED IS:

1. A process of administering to a mammal a retinoid campound which binds specifically or selectively to a RAR_α retinoid receptors in preference over RAR_β and RAR_Γ retinoid receptors, for the purpose of treating or preventing a disease or condition which is responsive to treatment by RAR_α specific or selective retinoid agonists.

2. A process in accordance with Claim 1 where the RAR_α specific or selective retinoid compound binds approximately 500 times stronger to RAR_α retinoid receptors than to RAR_β and RAR_Γ retinoid receptors.

3. A process in accordance with Claim 1 where the RAR_α specific or selective retinoid compound is administered to a mammal for the treatment or prevention of the disease or condition selected from acute monocytic leukemia, cervical carcinoma, myeloma, ovarian carcinomas, head and neck carcinomas, proliferative vitreoretinopathy (PVR) and age related macular degeneration (AMD).

4. A process in accordance with Claim 3 where the RAR_α specific or selective retinoid compound is administered in a dose of approximately 0.5 to 5 mg per kg body weight per day.

5. A process in accordance with Claim 1 where the RAR_α specific or selective retinoid compound is administered to a mammal for the treatment or prevention of the disease or condition selected from actinic keratoses, arsenic keratoses, inflammatory and non-inflammatory acne, psoriasis, ichthyoses, eczema, atopic dermatitis, Darriers disease, lichen planus, glucocorticoid damage, topical microbial infection, skin pigmentation, age and photo damage

1 to the skin, premalignant and malignant

2 hyperproliferative diseases, Kaposi's sarcoma,

3 diseases of the eye, proliferative vitreoretinopathy

4 (PVR), retinal detachment, dry eye and other

5 corneopathies, cardiovascular diseases,

6 dyslipidemias, prevention of post-angioplasty

7 restenosis, diseases associated with human papilloma

8 virus (HPV), inflammatory diseases,

9 neurodegenerative diseases, improper pituitary

10 function, insufficient hair growth, diseases

11 associated with the immune system, and wound

12 healing.

13   6. A process in accordance with Claim 1 where

14 the RAR_α specific or selective retnoid compound has

15 the formula (i) or the formula (ii)

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29   formula (i)   formula (ii)

30 where X_1 is O or X_1 is $[C(R_1)_2]_n$ where n is an integer

31 between 0 and 2;

32   R_1 is independently H or alkyl of 1 to 6

33 carbons;

34   R_2 is independently hydrogen, or lower alkyl of

1 to 6 carbons;

R₃ is hydrogen, lower alkyl of 1 to 6 carbons or F;

m is an integer having the value of 0 - 5;

o is an integer having the value of 0 - 4;

p is an integer having the value of 0 - 2;

r is an integer having the value 0 - 2;

X₂ is N or CH;

Y is a phenyl or naphthyl group, or heteroaryl selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl, thiazolyl, oxazolyl, imidazolyl and pyrrazolyl, said phenyl, naphthyl and heteroaryl groups being optionally substituted with one or two R₂ groups;

W₁ is a substituent selected independently from the group consisting of F, Br, Cl, I, fluoro substituted C_{1-6} alkyl, NO_2, and OH, with the provisos that:

(i) when the compound is in accordance with formula (i) and Z is O then the sum of p and r is at least 1 and W₁ is not a fluoro group in the 3 position of a tetrahydronaphthalene ring;

(ii) when the compound is in accordance with formula (i) and r is zero and p is 1 and W₁ is OH then the OH group is positioned α to the L group;

W₂ is a substituent selected independently from the group consisting of F, Br, Cl, I, fluoro substituted C_{1-6} alkyl, NO_2, and OH;

W₃ is a substituent selected independently from the group consisting of F, Br, Cl, I, C_{1-6}alkyl, fluoro substituted C_{1-6} alkyl, NO_2, and OH with the proviso that wh n the compound is in accordance with Formula 2 and X₂ is CH and r is 0 then p is not 0 and at least one W₃ group is not alkyl;

1   L is -(C=Z)-NH- or -NH-(C=Z)-   Z is O or S,

2   and

3   B is COOH or a pharmaceutically acceptable salt

4   thereof, $COOR_8$, $CONR_9R_{10}$, $-CH_2OH$, CH_2OR_{11}, CH_2OCOR_{11},

5   CHO, $CH(OR_{12})_2$, $CHOR_{13}O$, $-COR_7$, $CR_7(OR_{12})_2$, $CR_7OR_{13}O$,

6   where R_7 is an alkyl, cycloalkyl or alkenyl group

7   containing 1 to 5 carbons, R_8 is an alkyl group of 1

8   to 10 carbons or trimethylsilylalkyl where the alkyl

9   group has 1 to 10 carbons, or a cycloalkyl group of

10   5 to 10 carbons, or R_8 is phenyl or lower

11   alkylphenyl, R_9 and R_{10} independently are hydrogen,

12   an alkyl group of 1 to 10 carbons, or a cycloalkyl

13   group of 5-10 carbons, or phenyl or lower

14   alkylphenyl, R_{11} is lower alkyl, phenyl or lower

15   alkylphenyl, R_{12} is lower alkyl, and R_{13} is divalent

16   alkyl radical of 2-5 carbons.

17   7.   A process in accordance with Claim 6 where

18   the RAR_α specific or selective retinoid compound is

19   in accordance with formula (i).

20   8.   A process in accordance with Claim 7 where

21   in the formula of the RAR_α specific or selective

22   retinoid compound X_1 is $[C(R_1)_2]_n$ and n is 1.

23   9.   A process in accordance with Claim 8 where

24   in the formula of the RAR_α specific or selective

25   retinoid compound Y is phenyl.

26   10. A process in accordance with Claim 6 where

27   the RAR_α specific or selective retinoid compound is

28   in accordance with formula (ii).

29   11. A process in accordance with Claim 10 where

30   in the formula of the RAR_α specific or selective

31   retinoid compound Y is phenyl.

32   12. A process of administering to a mammal a

33   retinoid compound which binds specifically or
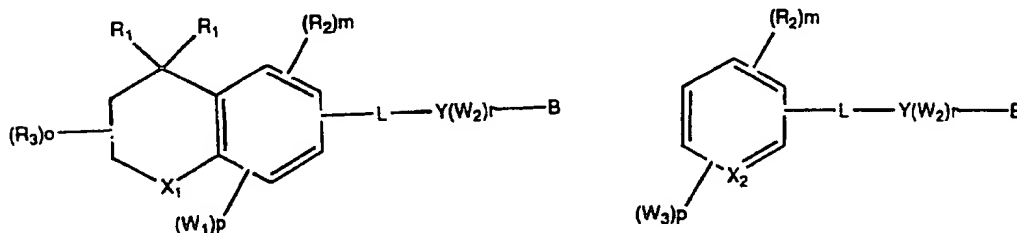
34   selectively to a RAR_α retinoid receptors in

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1   preference over RAR_β and RAR_Γ retinoid receptors, for
2   the purpose of treating or preventing a disease or
3   condition which is responsive to treatment by RAR_α
4   specific or selective retinoid agonists, the
5   retinoid compound being specific or selective for
6   RAR_α retinoid receptors in preference over RAR_β and
7   RAR_Γ retinoid receptors when in a binding assay the
8   K_d value of binding to RAR_α receptors is
9   approximately 500 times smaller than the K_d value for
10 binding to RAR_β and RAR_Γ retinoid receptors.

11   13. A process in accordance with Claim 12 where
12 the RAR_α specific or selective retinoid compound is
13 administered to a mammal for the treatment or
14 prevention of the disease or condition selected from
15 actinic keratoses, arsenic keratoses, inflammatory
16 and non-inflammatory acne, psoriasis, ichthyoses,
17 eczema, atopic dermatitis, Darriers disease, lichen
18 planus, glucocorticoid damage, topical microbial
19 infection, skin pigmentation, age and photo damage
20 to the skin, premalignant and malignant
21 hyperproliferative diseases, Kaposi's sarcoma,
22 diseases of the eye, proliferative vitreoretinopathy
23 (PVR), retinal detachment, dry eye and other
24 corneopathies, cardiovascular diseases,
25 dyslipidemias, prevention of post-angioplasty
26 restenosis, diseases associated with human papilloma
27 virus (HPV), inflammatory diseases,
28 neurodegenerative diseases, improper pituitary
29 function, insufficient hair growth, diseases
30 associated with the immune system, and wound
31 healing.

32   14. A process in accordanc with Claim 13 where
33 the RAR_α specific or selective retinoid compound is
34 administered to a mammal for the treatment or

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1 prevention of the disease or condition selected from

2 acute monocytic leukemia, cervical carcinoma,

3 myeloma, ovarian carcinomas, head and neck

4 carcinomas, proliferative vitreoretinopathy (PVR)

5 and age related macular degeneration (AMD).

6   15. A process in accordance with Claim 13 where

7 the RAR_α specific or selective retinoid compound has

8 the formula (i) or the formula (ii)

21   formula (i)   formula (ii)

22 where X_1 is O or X_1 is $[C(R_1)_2]_n$ where n is an integer

23 between 0 and 2;

24   R_1 is independently H or alkyl of 1 to 6

25 carbons;

26   R_2 is independently hydrogen, or lower alkyl of

27 1 to 6 carbons;

28   R_3 is hydrogen, lower alkyl of 1 to 6 carbons or

29 F;

30   m is an integer having the value of 0 – 5;

31   o is an integer having the value of 0 – 4;

32   p is an integer having the value of 0 – 2;

33   r is an integer having the value 0 – 2;

34   X_2 is N or CH;

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Y is a phenyl or naphthyl group, or heteroaryl
selected from a group consisting of pyridyl,
thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl,
thiazolyl, oxazolyl, imidazolyl and pyrrazolyl, said
phenyl, naphthyl and heteroaryl groups being
optionally substituted with one or two R_2 groups;

W_1 is a substituent selected independently from
the group consisting of F, Br, Cl, I, fluoro
substituted C_{1-6} alkyl, NO_2, and OH, with the provisos
that:

(i) when the compound is in accordance with
formula (i) and Z is O then the sum of p and r is at
least 1 and W_1 is not a fluoro group in the 3
position of a tetrahydronaphthalene ring;

(ii) when the compound is in accordance with
formula (i) and r is zero and p is 1 and W_1 is OH
then the OH group is positioned α to the L group;

W_2 is a substituent selected independently from
the group consisting of F, Br, Cl, I, fluoro
substituted C_{1-6} alkyl, NO_2, and OH;

W_3 is a substituent selected independently from
the group consisting of F, Br, Cl, I, C_{1-6}alkyl,
fluoro substituted C_{1-6} alkyl, NO_2, and OH with the
proviso that when the compound is in accordance with
Formula 2 and X_2 is CH and r is 0 then p is not 0 and
at least one W_3 group is not alkyl;

L is -(C=Z)-NH- or -NH-(C=Z)-

Z is O or S, and

B is COOH or a pharmaceutically acceptable salt
thereof, $COOR_8$, $CONR_9R_{10}$, $-CH_2OH$, CH_2OR_{11}, CH_2OCOR_{11},
CHO, $CH(OR_{12})_2$, $CHOR_{13}O$, $-COR_7$, $CR_7(OR_{12})_2$, $CR_7OR_{13}O$,
where R_7 is an alkyl, cycloalkyl or alk nyl group
containing 1 to 5 carbons, R_8 is an alkyl group of 1
to 10 carbons or trimethylsilylalkyl where the alkyl

1   group has 1 to 10 carbons, or a cycloalkyl group of

2   5 to 10 carbons, or R_8 is phenyl or lower

3   alkylphenyl, R_9 and R_{10} independently are hydrogen,

4   an alkyl group of 1 to 10 carbons, or a cycloalkyl

5   group of 5-10 carbons, or phenyl or lower

6   alkylphenyl, R_{11} is lower alkyl, phenyl or lower

7   alkylphenyl, R_{12} is lower alkyl, and R_{13} is divalent

8   alkyl radical of 2-5 carbons.

9   16. A process in accordance with Claim 15 where

10   the RAR_α specific or selective retinoid compound is

11   in accordance with formula (i).

12   17. A process in accordance with Claim 15 where

13   the formula the RAR_α specific or selective retinoid

14   compound is in accordance with formula (ii).

15   18. A process of administering to a mammal a

16   retinoid compound which binds specifically or

17   selectively to a RAR_α retinoid receptors in

18   preference over RAR_β and RAR_Γ retinoid receptors, for

19   the purpose of treating or preventing the disease or

20   condition selected from acute monocytic leukemia,

21   cervical carcinoma, myeloma, ovarian carcinomas,

22   head and neck carcinomas, proliferative

23   vitreoretinopathy (PVR) and age related macular

24   degeneration (AMD) the retinoid compound being

25   specific or selective for RAR_α retinoid receptors in

26   preference over RAR_β and RAR_Γ retinoid receptors when

27   in a binding assay the K_d value of binding to RAR_α

28   receptors is approximately 500 times smaller than

29   the K_d value for binding to RAR_β and RAR_Γ retinoid

30   receptors, the retinoid compound having the formula

31   (i) or the formula (ii)

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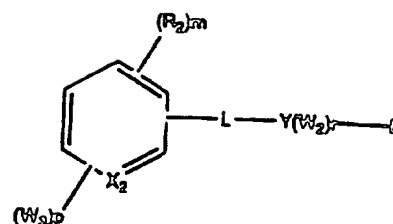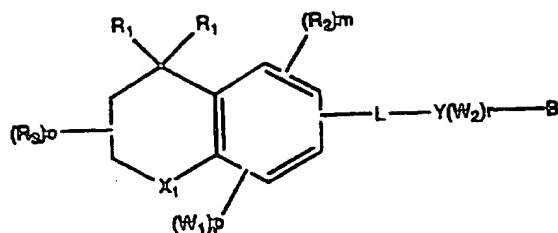
        formula (i)   formula (ii)

where X_1 is O or X_1 is $[C(R_1)_2]_n$ where n is an integer
between 0 and 2;

        R_1 is independently H or alkyl of 1 to 6
carbons;

        R_2 is independently hydrogen, or lower alkyl of
1 to 6 carbons;

        R_3 is hydrogen, lower alkyl of 1 to 6 carbons or
F;

        m is an integer having the value of 0 - 5;

        o is an integer having the value of 0 - 4;

        p is an integer having the value of 0 - 2;

        r is an integer having the value 0 - 2;

        X_2 is N or CH;

        Y is a phenyl or naphthyl group, or heteroaryl
selected from a group consisting of pyridyl,
thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl,
thiazolyl, oxazolyl, imidazolyl and pyrrazolyl, said
phenyl, naphthyl and heteroaryl groups being
optionally substituted with one or two R_2 groups;

        W_1 is a substituent selected independently from
the group consisting of F, Br, Cl, I, fluoro
substituted C_{1-6} alkyl, NO_2, and OH, with the provisos
that:

        (i)   when the compound is in accordance with

formula (i) and Z is O then the sum of p and r is at least 1 and W_1 is not a fluoro group in the 3 position of a tetrahydronaphthalene ring;

(ii) when the compound is in accordance with formula (ii) and r is zero and p is 1 and W_1 is OH then the OH group is positioned α to the L group;

W_2 is a substituent selected independently from the group consisting of F, Br, Cl, I, fluoro substituted C_{1-6} alkyl, NO_2, and OH;

W_3 is a substituent selected independently from the group consisting of F, Br, Cl, I, C_{1-6}alkyl, fluoro substituted C_{1-6} alkyl, NO_2, and OH with the proviso that when the compound is in accordance with Formula 2 and X_2 is CH and r is 0 then p is not 0 and at least one W_3 group is not alkyl;

L is $-(C=Z)-NH-$ or $-NH-(C=Z)-$

Z is O or S, and

B is COOH or a pharmaceutically acceptable salt thereof, $COOR_8$, $CONR_9R_{10}$, $-CH_2OH$, CH_2OR_{11}, CH_2OCOR_{11}, CHO, $CH(OR_{12})_2$, $CHOR_{13}O$, $-COR_7$, $CR_7(OR_{12})_2$, $CR_7OR_{13}O$, where R_7 is an alkyl, cycloalkyl or alkenyl group containing 1 to 5 carbons, R_8 is an alkyl group of 1 to 10 carbons or trimethylsilylalkyl where the alkyl group has 1 to 10 carbons, or a cycloalkyl group of 5 to 10 carbons, or R_8 is phenyl or lower alkylphenyl, R_9 and R_{10} independently are hydrogen, an alkyl group of 1 to 10 carbons, or a cycloalkyl group of 5-10 carbons, or phenyl or lower alkylphenyl, R_{11} is lower alkyl, phenyl or lower alkylphenyl, R_{12} is lower alkyl, and R_{13} is divalent alkyl radical of 2-5 carbons.

19. A process in accordance with Claim 18 where the RAR_α specific or selective retinoid compound is in accordance with formula (i), and Y is phenyl.

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1   20. A process in accordance with Claim 19 where the

2   RAR$_\alpha$ specific or selective retinoid compound is

3   selected from the group consisting of:

4   ethyl 2-fluoro-4-[(5',6',7',8'-tetrahydro-

5   5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]be

6   nzoate;

7   2-fluoro-4-[(5',6',7',8'-tetrahydro-

8   5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]be

9   nzoic acid;

10   ethyl 2-fluoro-4-[(5',6',7',8'-tetrahydro-4'-

11   bromo-5',5',8',8'-tetramethylnaphthalen-2'-yl)carbam

12   oyl]benzoate;

13   2-fluoro-4-[(4'-bromo-5',6',7',8'-tetrahydro-

14   5',5',8',8'-tetramethylnaphthalen-2'-yl)carbamoyl]be

15   nzoic acid;

16   ethyl

17   2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-bromochroman

18   -6'-yl)carbamoyl]benzoate;

19

20   2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-bromochroman

21   - 6'-yl)carbamoyl]benzoic acid;

22   ethyl 2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-

23   trifluoromethylchroman-6'-yl)carbamoyl] benzoate;

24   2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-

25   trifluoro-methylchroman-6'-yl)carbamoyl] benzoic

26   acid;

27   ethyl 2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-

28   azidochroman-6'-yl)carbamoyl]benzoate;

29   2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-

30   azidochroman- 6'-yl)carbamoyl]benzoic acid;

31   ethyl 2-fluoro-4-[(2', 2', 4', 4'-tetramethyl-

32   8'-iodochroman-6'-yl)carbamoyl]benzoat ;

33   2-fluoro-4-[(2',2',4',4'-tetramethyl-8'-

34   iodochroman-6'-yl)carbamoyl]benzoic acid;

ethyl 4-[(5',6',7',8'-tetrahydro-5',5',8',8'-
tetramethyl-2-naphthalenyl)thiocarbamoyl]benzoate,
and

4-[(5',6',7',8'-tetrahydro-5',5',8',8'-
tetramethylnaphthalen-2'-yl)thiocarbamoyl]benzoic
acid.

21. A process in accordance with Claim 18 where
the RAR$_\alpha$ specific or selective retinoid compound is
in accordance with formula (ii), and Y is phenyl.

22. A process in accordance with Claim 19 where
the RAR$_\alpha$ specific or selective retinoid compound
is:

ethyl 2-fluoro-4-[(2'6'-di-tert-butylpyrid-4'-
yl)carbamoyl]benzoate, or

2-fluoro-4-[(2',6'-di-t-butylpyrid-4'-
yl)carbamoyl]benzoic acid.

1/4
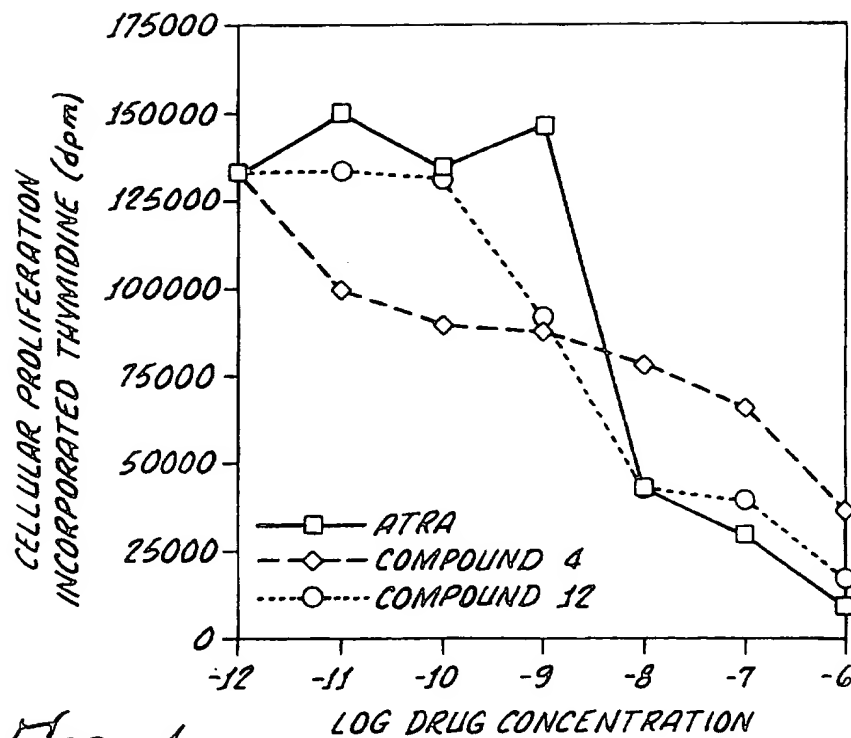
FIG. 1.
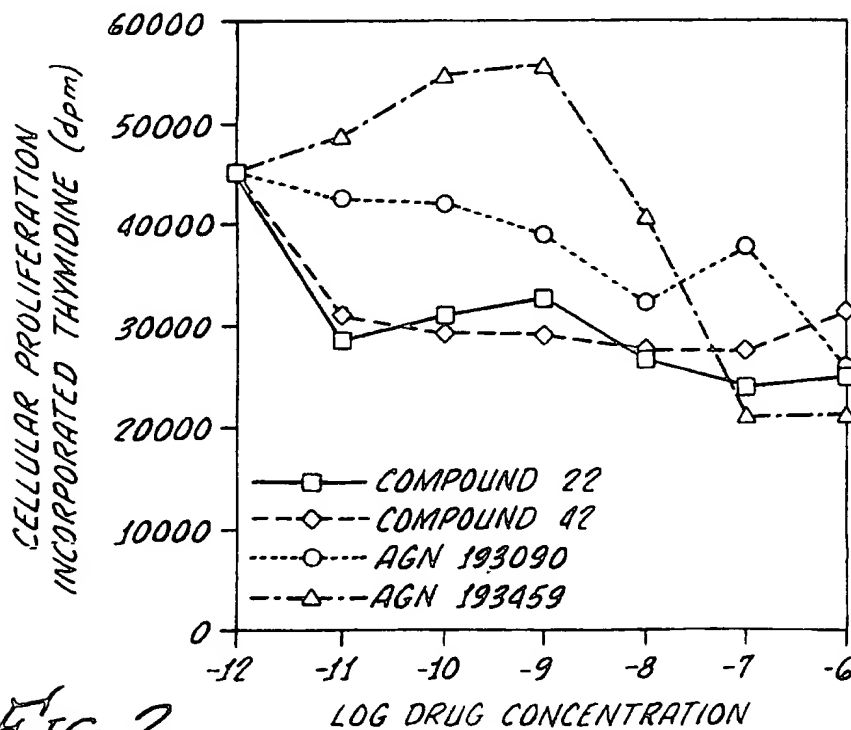
FIG. 2.
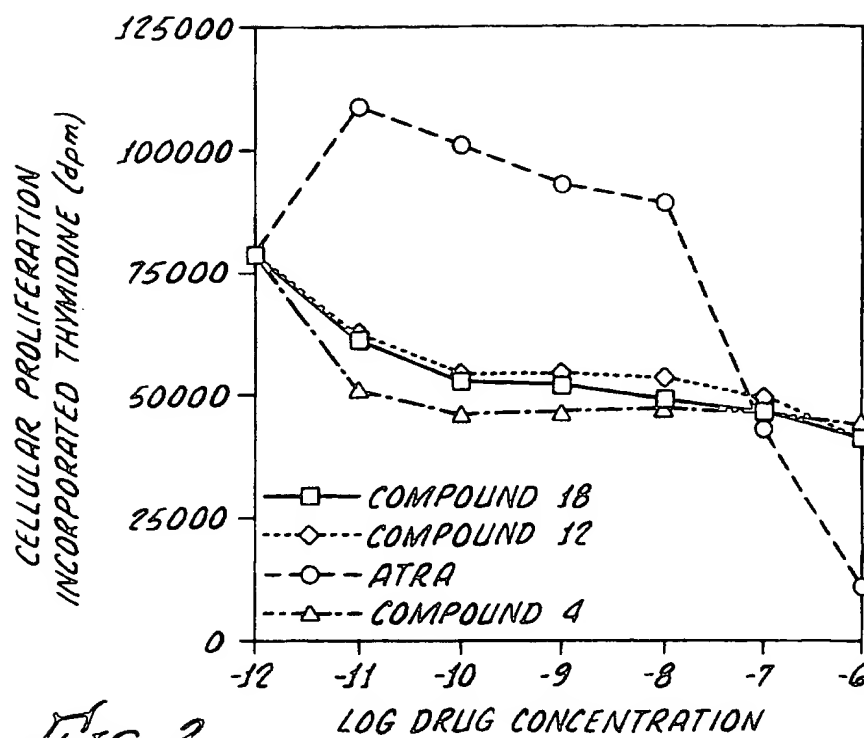
FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

_FIG. 7.

_FIG. 8.

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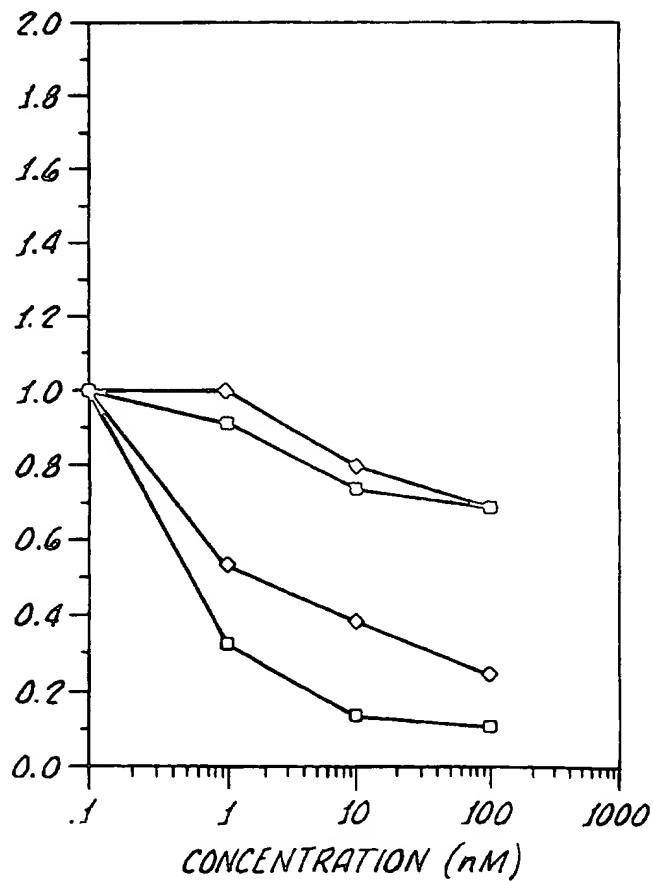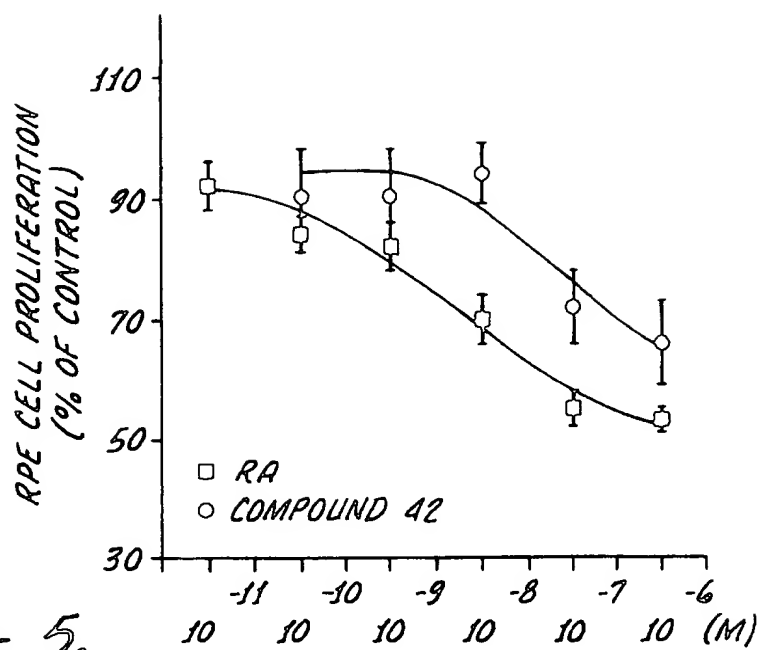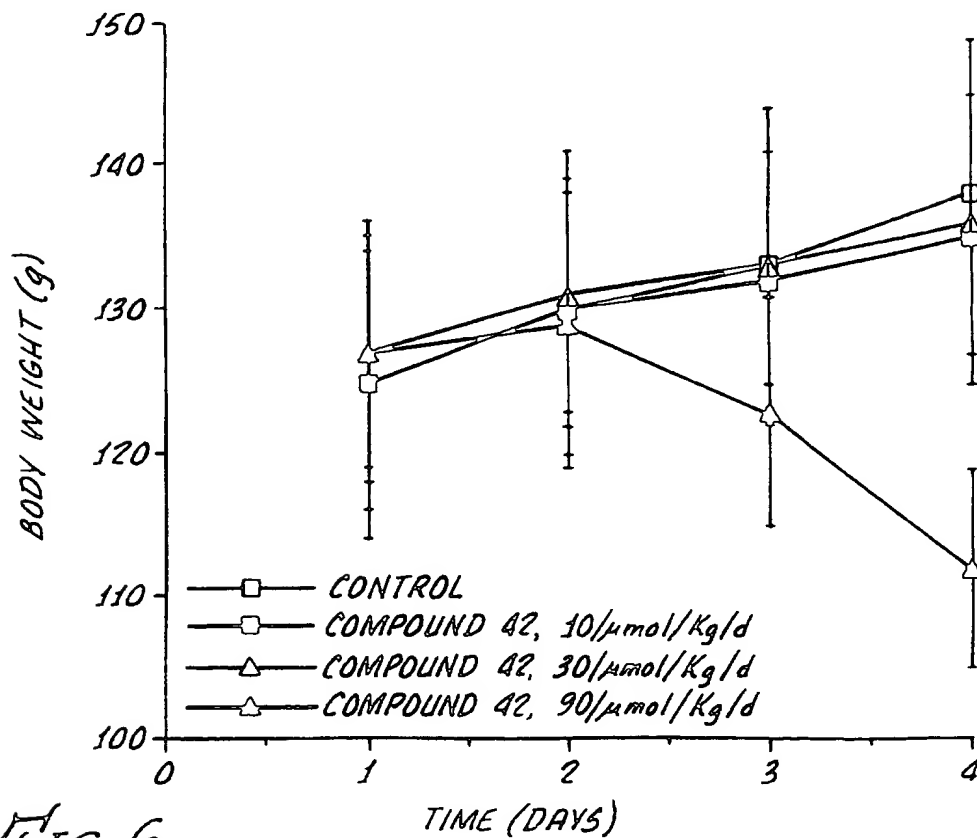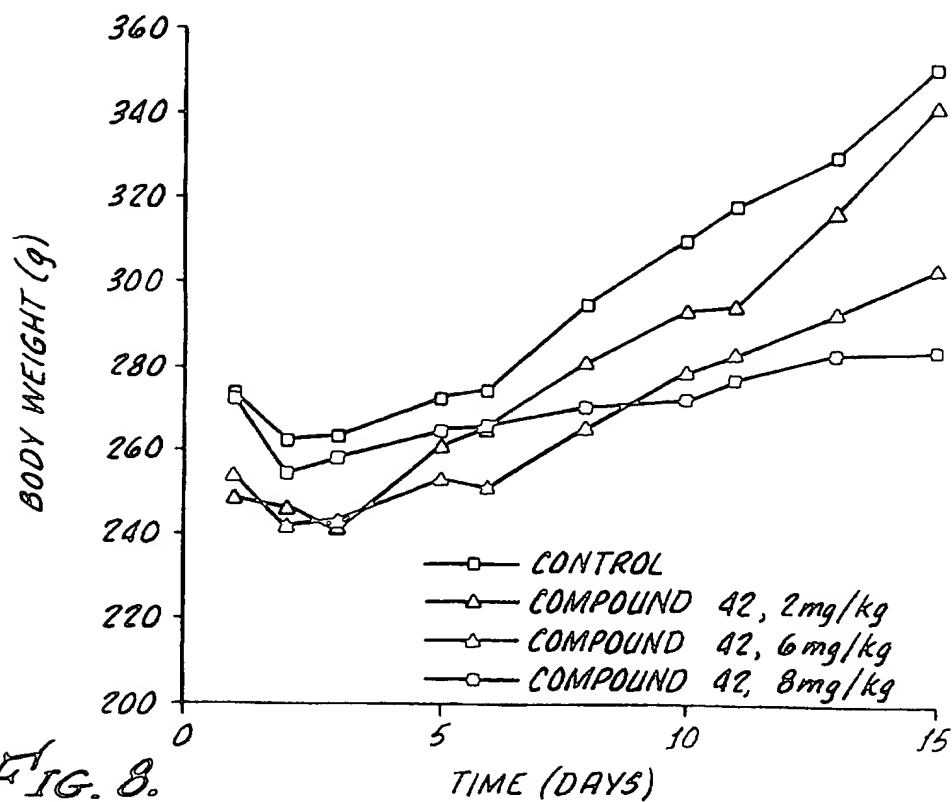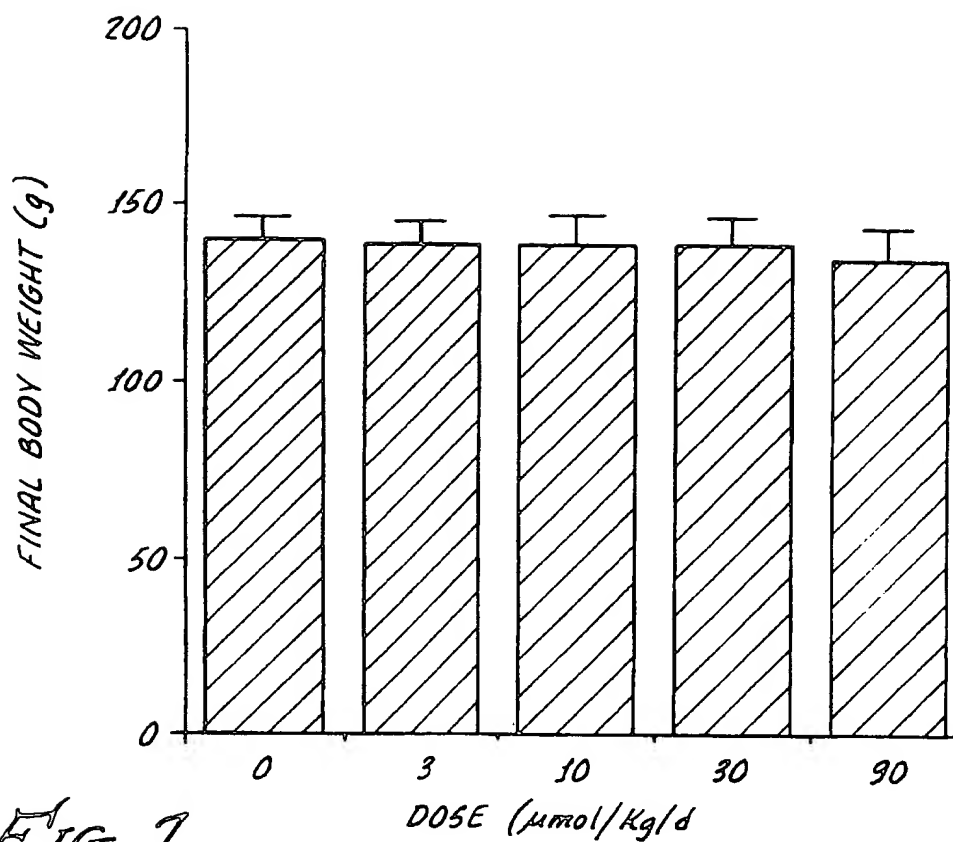
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : A61K 31/19, 31/215, 31/34, 31/44	A3	(11) International Publication Number: WO 97/24116
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(74) Agents: BARAN, Robert, J. et al.; Allergan, Inc., 2525 Dupont Drive. T-2,2-E, P.O. Box 19534, Irvine, CA 92623-9534 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
13 November 1997 (13.11.97)

(54) Title: METHODS OF TREATMENT WITH COMPOUNDS HAVING RARα RECEPTOR SPECIFIC OR SELECTIVE ACTIVITY

(57) Abstract

Retinoid compounds which act specifically or selectively on RARα receptor subtypes in preference over RARβ and RARτ receptor subtypes, possess desirable pharmaceutical properties associated with retinoids, and are particularly suitable for treatment of tumors, such as acute monocytic leukemia, cervical carcinoma, myeloma, ovarian carcinomas and head and neck carcinomas, without having one or more undesirable side effects of retinoids, such as inducement of weight loss, mucocutaneous toxicity, skin irritation and teratogenicity.

Intern   ial Application No

PCT/US 96/20511

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61K31/19   A61K31/215   A61K31/34   A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 03713 A (SALK INST FOR BIOLOGICAL STUDI) 4 March 1993 see page 9, line 4 - line 31; claims 1-15 --- -/--	1-4, 6-12, 14-22

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 1997	30.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Seegert, K

Form PCT/ISA/210 (second sheet) (July 1992)

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 121, no. 9, 1994 Columbus, Ohio, US; abstract no. 108128d, XP002030814 see abstract & YU, BAOXIN ET AL: "Synthesis of p-substituted benzoylaminobenzoic acid (methyl esters) and its differentiation induction activities of human promyelocytic leukemia cells HL-60" HUAXI YIKE DAXUE XUEBAO, vol. 25, no. 1, 1994, pages 30-34, ---	1-4, 6-12, 14-22
Y	DATABASE WPI Section Ch, Week 9416 Derwent Publications Ltd., London, GB; Class B05, AN 94-128759 XP002030803 & JP 06 072 866 A (CHUGOKU IGAKUKAGAKUIN YAKUBUTSU KENKYUSH) , 15 March 1994 see abstract ---	1-4, 6-12, 14-22
Y	EP 0 514 269 A (CIRD GALDERMA) 19 November 1992 see page 3, line 3 - page 3, line 9; claims 1-21 especially claim 16, ex. 15 ---	1-4, 6-12, 14-22
Y	EP 0 350 846 A (HOFFMANN LA ROCHE) 17 January 1990 see page 5, line 43 - page 6, line 35; claims 1-18 ---	1-4, 6-12, 14-22
Y	US 5 420 145 A (SHUDO KOICHI) 30 May 1995 see column 3, line 65 - column 4, line 29; claims 1-4 ---	1-4, 6-12, 14-22
Y	EP 0 170 105 A (SHUDO KOICHI ;SUMITOMO PHARMA (JP); YOSHITOMI PHARMACEUTICAL (JP)) 5 February 1986 see claims 1-11 ---	1-4, 6-12, 14-22
P,Y	WO 96 32101 A (TAIHO PHARMACEUTICAL CO LTD ;SHIBATA JIRO (JP); WIERZBA KONSTANTY) 17 October 1996 see abstract --- -/--	1-4, 6-12, 14-22

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAGECHIKA H ET AL: "RETINOBENZOIC ACIDS STRUCTURE-ACTIVITY RELATIONSHIPS OF AROMATIC AMIDES WITH RETINOIDAL ACTIVITY" JOURNAL OF MEDICINAL CHEMISTRY, vol. 31, no. 11, November 1988, pages 2182-2192, XP000608417 see tables I-VI see page 2187, left-hand column, last paragraph - page 2188, left-hand column	1-4, 6-12, 14-22
P,Y	MIN TENG ET AL: "Identification of a Retinoic Acid Receptor alpha Subtype Specific Agonist" J. MED. CHEM., vol. 39, no. 16, 2 August 1996, pages 3035-3038, XP000652115 see the whole document	1-4, 6-12, 14-22

1

Box I   Observations where certain claims were found unsearchable (Continuation of Item 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

> See annex.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

> 1-4,6-12, 14-22 (partially)

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                            ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION C NTINUED FROM   PCT/ISA/210

1.   The use of a RAR alpha selective agonist for the prevention/treatment of acute monocytic leukemia, cervical carcinoma, myeloma, ovarian carcinomas, head and neck carcinomas (respective portions of Claims 1- 4, 6-12, 14 - 22)

2.   The use of a RAR alpha selective agonist for the prevention/treatment of proliferative vitreoretinopathy (PVR), age related macular degeneration (AMD), diseases of the eye, retinal detachment, dry eye, other corneopathies (respective portions of Claims 1 - 22)

3.   The use of a RAR alpha selective agonist for the prevention/treatment of actinic keratoses, arsenic keratoses, inflammatory and non-inflammatory acne, psoriasis, ichtyoses, eczema, atopic dermatitis, Darriers disease, lichen planus, skin pigmentation, age and photo damage to the skin, premalignant and malignant hyperproliferative diseases, Kaposi's sarcoma (respective portions of Claims 1, 2, 4 - 13, 15- 22)

4.   The use of a RAR alpha selective agonist for the prevention/treatment of cardiovascular diseases (respective portions of Claims 1, 2, 4 - 13, 15- 22)

5.   The use of a RAR alpha selective agonist for the prevention/treatment of dyslipidemias (respective portions of Claims 1, 2, 4 - 13, 15- 22)

6. The use of a RAR alpha selective agonist for the prevention of post-angioplasty restenosis (respective portions of Claims 1, 2, 4 - 13, 15- 22)

INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/210

7.   The use of a RAR alpha selective agonist for the
prevention/treatment of diseases associated with human
papilloma virus (HPV) (respective portions of Claims 1,
2, 4 - 13, 15- 22)

8.   The use of a RAR alpha selective agonist for the
for the prevention/treatment of inflammatory diseases
(respective portions of Claims 1, 2, 4 - 13, 15- 22)

9. The use of a RAR alpha selective agonist for the
prevention/treatment of neurodegenerative diseases (re-
spective portions of Claims 1, 2, 4 - 13, 15- 22)

10.   The use of a RAR alpha selective agonist for the
prevention/treatment of improper pituitary function (re-
spective portions of Claims 1, 2, 4 - 13, 15- 22)

11. The use of a RAR alpha selective agonist for the
prevention/treatment of insufficient hair growth (re-
spective portions of Claims 1, 2, 4 - 13, 15- 22)

12. The use of a RAR alpha selective agonist for the
prevention/treatment of diseases associated with the im-
mune system (respective portions of Claims 1, 2, 4 - 13,
15- 22)

13. The use of a RAR alpha selective agonist for wound
healing (respective portions of Claims 1, 2, 4 - 13, 15-
22)

The search has been limited to the subject-matter of item 1.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9303713 A	04-03-93	AU 2516092 A CA 2114936 A EP 0600028 A JP 7505607 T US 5668175 A	16-03-93 04-03-93 08-06-94 22-06-95 16-09-97
EP 0514269 A	19-11-92	FR 2676440 A AT 129698 T AU 650972 B AU 1627392 A CA 2068696 A DE 69205725 D DE 69205725 T ES 2080458 T JP 5221951 A	20-11-92 15-11-95 07-07-94 19-11-92 16-11-92 07-12-95 30-05-96 01-02-96 31-08-93
EP 0350846 A	17-01-90	AT 128974 T AU 626881 B AU 3709789 A CA 1319364 A DE 58909463 D ES 2078905 T FI 96204 B IE 70450 B IL 90912 A JP 2053684 C JP 2076862 A JP 7086094 B PT 91158 B US 5420273 A US 5037825 A US 5164387 A US 5300522 A MX 16751 A	15-10-95 13-08-92 18-01-90 22-06-93 16-11-95 01-01-96 15-02-96 27-11-96 25-01-94 23-05-96 16-03-90 20-09-95 01-03-95 30-05-95 06-08-91 17-11-92 05-04-94 01-10-93
US 5420145 A	30-05-95	EP 0617020 A	28-09-94
EP 0170105 A	05-02-86	JP 1764534 C JP 4058458 B JP 61022047 A	28-05-93 17-09-92 30-01-86

Form PCT/ISA/210 (patent family annex) (July 1992)

page 1 of 2

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0170105 A		JP 1764542 C	28-05-93
		JP 4058459 B	17-09-92
		JP 61076440 A	18-04-86
		US 4703110 A	27-10-87
WO 9632101 A	17-10-96	AU 5287596 A	30-10-96
		CA 2191850 A	17-10-96
		EP 0768084 A	16-04-97